PEDIATRIC CLINICS OF NORTH AMERICA

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FOREWORD

WOLF W. ZUELZER, M.D.

Consulting Editor

It is tempting to limit these introductory remarks to an expression of gratitude on the part of the Consulting Editor to the authors whose ideas and knowledge have made this symposium possible and who gave generously of their experience and their time. One may, indeed, be encouraged to think that this volume will speak for itself. A compact source of information about hematological disorders of childhood is obviously needed at this time, and the caliber of the contributors is the best guarantee for its quality. If any foreword is justified, it is only because in a sense this symposium is a "first."

Hematology, pediatric and otherwise, has grown so rapidly in the last decade that the technical complexities at times may seem to outweigh the gains made in basic physiologic knowledge on which clinical practice must be based. In reality, the progress has been largely in the direction of physiology and of simplification. One might almost say that the field has become a highly developed area of applied physiology. The era in which hematology was chiefly the province of the morphologist describing with meticulous care the characteristics of the individual blood cells is past. The morphologist is needed as much as ever, but he is now a member of a team on which the immunologist, the biochemist, the nutritionist, the geneticist and many others are equally important.

And yet the accomplishments of these specialists make the application of this new knowledge easier rather than more complex for the physician who is responsible for the care of the patient. They contribute clarity where there was obscurity, measurement where there were semantics. Many of the new concepts in hematology are ripe for clinical use. The gap between investigation and practice is ready to be bridged.

No one is better qualified for this task than the investigators them-

selves, who combine clinical experience with experimental work. Moreover, everyone contributing to this volume is either a pediatrician himself or has had ample exposure to pediatric problems so that the special aspects of pediatric hematology could receive expert attention.

No attempt has been made to "cover the waterfront." Even so, a fairly complete list of topics is covered. No attempt has been made, either, to impose any uniformity of viewpoint. Each contributor has presented his own views and conclusions on the basis of his own experience. If at times there are discrepancies, an additional purpose will have been served: that of illustrating that knowledge in a growing, dynamic field such as hematology is still in flux, that there is no such thing as absolute truth, and that a grain or two of salt are as essential an ingredient for the reader as they are for the author.

THE GENETICS OF BLOOD

JAMES V. NEEL, M.D.

At this writing there appear to be more clearly and simply inherited traits recognized for blood than for any other tissue of the body, with the possible exception of cutaneous tissue. This, in all probability, does not imply a greater genetic variability on the part of the component cells and the surrounding medium of this tissue, but rather reflects the relative ease with which blood can be studied. No other tissue in the body can be so readily biopsied, and its various aspects so conveniently subjected to a searching examination. It seems quite likely that, within reasonable limits, in time, and with the development of new techniques such as those described by Puck and his associates,[32, 33] we shall recognize a range of genetic variation for other tissues comparable to that already known for this one. In this sense, then, the findings we shall briefly review serve as a model for the kind of genetic data which in the foreseeable future will exist for all the tissues of the body, although it may be taken as an article of faith that even this relatively advanced model is still in the early stages of its final form. No attempt at complete documentation will be made in the following account, but key references will be given in each section.

BASIC GENETIC PRINCIPLES

The principles which underlie the transmission of the characteristics to be discussed are in essence extremely simple. The inherited traits of man have as their ultimate basis the nucleoproteins of the 48 chromosomes.* The chromosomes are differentiated along their lengths into specific subdivisions known as *genes*, each gene being associated with a particular hereditary effect. The site on a chromosome at which a particular gene occurs is known as a *locus*. Although at one time it was customary to think of the genes as rather well delineated subdivisions,

* Tjio[44] finds that there are only 46 chromosomes in human cells.

the current trend is to think of each chromosome as a continuum, a collection of tremendously elongate nucleic acid strands, the different "genes" being variations in the precise sequence of the constituents of the nucleic acid strands in a particular area of the chromosome. Just as every individual possesses two of every different kind of chromosome, one member of the pair derived from the father and one from the mother, so he possesses two representatives of each kind of gene. If the two members of a gene pair differ in their attributes, and if the effects of one member of the pair are apparent in an individual to the seeming exclusion of the other, then the gene that finds obvious expression is termed a *dominant* gene. The gene whose effects are apparently hidden is termed a *recessive* gene; such genes find expression only when present in both the members of a pair of chromosomes. When the members of a gene pair are alike, there is *homozygosity* at that particular genetic locus; when they are unlike, there is *heterozygosity*. The various alternative forms of a gene which may occur at a given locus on a chromosome are known as *alleles*. With respect to some loci, as many as a dozen alleles are known, no more than two of which, of course, can be present in an individual. The term *genotype* refers to the genetic constitution of an individual, either *in toto* or with respect to one or several traits. The term *phenotype* by contrast refers to the appearance of an individual as determined both by genotype and environmental factors.

In the course of the maturation divisions which precede the formation of the gametes, each sperm or egg cell receives one or the other member of each chromosome pair and accordingly one or the other representative of each gene pair. Thus, with respect to any particular gene present in an individual, half of that individual's gametes, and half of his children, will, on the average, receive that particular gene. But unless a similar gene is contributed by the other parent, the children will exhibit the effects associated with that gene only if it is a dominant. During the first maturation division the members of a pair of chromosomes become closely united along their lengths; during this pairing there may be an actual exchange of segments between the chromosomes, termed *crossing-over*.

Figure 1 is a diagrammatic presentation of dominant inheritance. When a person with disease due to a dominant gene marries a normal person, half of the children, irrespective of their sex, will be expected to exhibit the condition in question, while half of the children will be normal. If the affected children marry normal persons, half of their children will in turn be affected, and half normal. The trait does not reappear among the offspring of the normal children of affected individuals. If by chance two people with the same dominantly inherited trait marry, on the average one quarter of their children may receive

the dominant gene from both parents; such children in consequence usually exhibit a much more extreme departure from normality than either parent.

In recessive inheritance, on the other hand, although affected children may arise from marriages involving one or two affected parents, usually they result from marriages in which both parents appear normal. In such marriages both parents carry hidden (recessive) determiners for the trait in question in their genetic make-up. When two parents carry

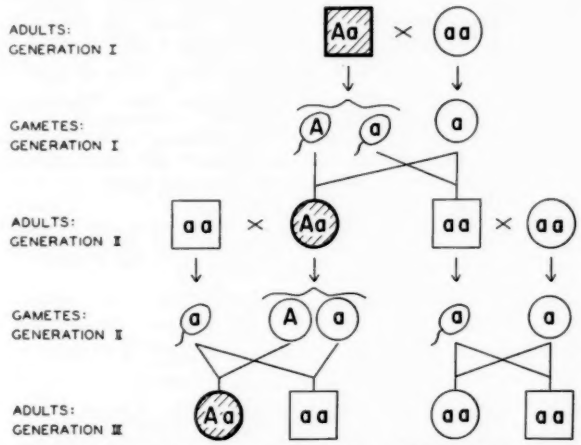

Fig. 1. The mechanism of dominant, nonsex-linked inheritance. It is assumed that the gene "A" determines a pathological trait and that this gene is dominant to its normal allele, designated by "a." Individuals who would be expected to demonstrate the trait determined by "A" are shown by heavy, obliquely hatched symbols. Further explanation in text. (By permission from *Human Heredity*, by J. V. Neel and W. J. Schull, copyright 1954, University of Chicago.)

the same recessive gene, the probability is one in four that any particular child of theirs will be homozygous for that gene and so exhibit the trait for which the gene is responsible, while the probability is one in two that the child, like the parents, will appear normal, but be heterozygous for the gene. Finally, the probability is one in four that the child will not carry the gene at all. A marriage of this type is diagrammed in Figure 2. As noted above, children affected with a recessive trait may arise in two other ways, namely, (1) from a marriage between a person with a recessive trait and an apparently normal person who is heterozygous for the same gene, with half of the children being affected, or (2) from a marriage of persons both with the same recessive trait, with all the children affected. These two types of marriages are also diagrammed in Figure 2.

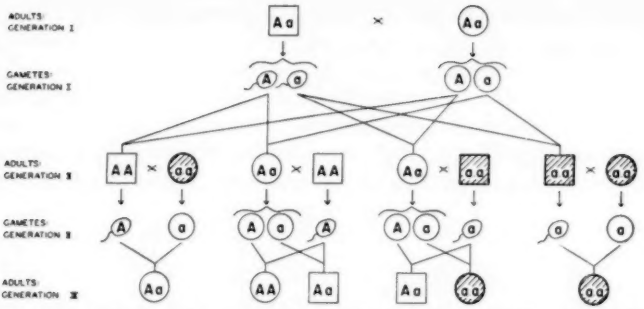

Fig. 2. The mechanism of recessive nonsex-linked inheritance. Although individuals exhibiting recessive traits may arise from a variety of marriage types, the most usual situation with respect to a rare recessive trait is the mating shown first in the figure. Two normal-appearing individuals, both heterozygous for the same pathological recessive gene, here "a," marry. The possible results from the random union of the two types of sperm with the two types of eggs are as diagrammed. Also diagrammed are certain further types of marriage. Affected individuals are indicated by the heavy, obliquely hatched symbols. Further explanation in text. (By permission from *Human Heredity*, by J. V. Neel and W. J. Schull, copyright 1954, University of Chicago.)

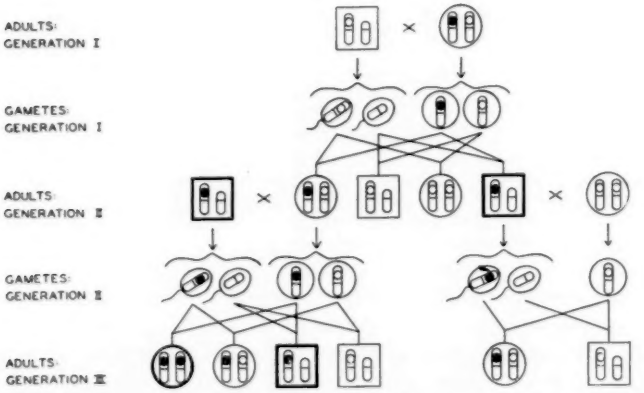

Fig. 3. Recessive, sex-linked inheritance. The X chromosome is the longer of the two chromosomes depicted. The recessive, pathological gene is identified by a black dot. Individuals who would be expected to exhibit the trait in question are shown by the heavy symbol. Three types of marriage are shown. The first indicates the most common situation, of a female carrier producing an affected son. The second illustrates how females exhibiting a recessive, sex-linked trait may arise from the marriage of an affected male and a carrier female. The third shows how all the daughters of an affected male are carriers. (By permission from *Human Heredity*, by J. V. Neel and W. J. Schull, copyright 1954, University of Chicago.)

One special category of recessively inherited disease is known as *sex-linked*. There is an important exception to the rule that the chromosomes occur in pairs which, although they may differ in detailed genetic content, are in general more or less similar. The occurrence of the two sexes in approximately equal numbers depends upon the fact that the male has an unequal pair of chromosomes, known as the X and Y chromosomes, whereas the female has two X's. The Y chromosome is relatively inactive genetically. The male forms two types of sperm, half X-bearing, half Y-bearing, which, uniting with X-bearing eggs, result in XY zygotes (which develop into males) and XX zygotes (which develop into females). Since the male has only a single X chromosome, recessive genes located on the X, such as those responsible for hemophilia, may find visible expression in the male when represented only once in the genotype, but must be homozygous in females to find expression. Figure 3 is a diagrammatic presentation of the behavior of recessive sex-linked genes.

Genetics of Blood Cells

With respect to most of the genes responsible for variations in the blood cells, we do not encounter simple dominance or recessiveness, but an intermediate type of inheritance, such that when two alleles are present, each finds phenotypic expression. Indeed, the finding that so many of the mutant genes affecting blood cell characteristics fail to exhibit simple recessiveness, but have some effects when heterozygous, has done much to undermine the concept of complete recessivity, and, by extrapolation, to make it probable that most mutant genes find *some* expression when present. Each year our ability to detect these heterozygote manifestations is improving.

It is in this connection interesting to consider the relation of sequence of discovery to what is considered dominant and what recessive. This is nicely illustrated by the history of the Kell-Cellano blood groups. The Kell antibody was discovered in the serum of a woman who had given birth to a child with hemolytic disease. This antibody was found to agglutinate the red cells of some 9 per cent of western Europeans; family studies suggested that the presence of the corresponding red cell antigen was determined by a single gene, *K*, with the antigen formed by persons either homozygous or heterozygous for this gene. By definition, then, since the gene finds expression when heterozygous, it should be classified as a dominant, and its allelomorph, non-Kell, was designated as *k*. But a few years after the discovery of the Kell antibody, another antibody was discovered, also in the serum of a woman who had given birth to a child with mild hemolytic disease, which agglutinated the cells of all persons except those whose genotype was *KK*.

This new antibody was, in fact, capable of identifying an antigen produced by the *k* gene, no matter whether the gene was homozygous (*kk*) or heterozygous (*Kk*). Had this second antibody been discovered first, it would have been proper to designate the responsible gene as, e.g., *C* and its allele as *c*. The point is that neither of these genes is dominant to the other, but each is responsible, when homozygous or heterozygous, for the production of a characteristic antigen which can be identified by the appropriate antiserum. This type of situation is characteristic of many of the blood groups.

A more extended account of genetic principles with particular reference to man will be found in the books of Stern[42] and Neel and Schull.[26]

THE GENETICS OF THE RED BLOOD CELL

At first glance it would appear to be a paradox that the simplest cell of the body, in terms of its apparent morphological complexity, is the one concerning whose genetic variations we know the most. As pointed out in the introduction, this undoubtedly reflects the ease with which this cell type can be studied, and the corresponding amount of attention and variety of technical approaches that have been lavished upon it. For purposes of convenience we shall consider the known genetic variations under four headings: namely, (1) serotypes, reflecting primarily variations in the cell envelope, (2) hemoglobin types, (3) morphological types, apparently involving "stromal" differences, and (4) enzyme types. It must be recognized that these are subdivisions of convenience rather than of morphology or function in the strict sense; the reader desiring a more sophisticated view of the structure of the red cell is referred to the review by Ponder.[29]

Serotypes

Even to the initiated the details of serological genetics may be confusing. The basic facts are not particularly complicated, but these have often been obscured by the terminological problems which arise when, in the tempo of rapid discovery, a system of terminology grows more by accretion than by design. Indeed, it may be said that the terminological problems raised by the blood groups have done more than any other developments in human genetics to underline the need for devising elastic and readily modifiable genetic terminologies. While it is obviously impossible to predict discoveries, at the very least we can strive for a terminology that leaves room for the later discoveries that can be confidently anticipated.

Two blood group systems are of particular importance as a cause of

hemolytic disease of the newborn, and it is to these that most of our attention will be directed.

THE A-B-O SYSTEM. The four blood groups, AB, A, B and O, are determined by a series of three allelomorphs. These are designated as A, B and O. The A and B genes appear to be dominant to the O gene, but do not show dominance with respect to one another, so that a person of genotype AB has both A and B antigens in his erythrocytes. To complete the story, as is well known, anti-A and anti-B agglutinins occur naturally in serum, in reciprocal relationship to the red cell antigens, as shown in Table 1. Table 2 diagrams the various possible types of marriage with respect to these three genes, the marriages grouped according to phenotype.

TABLE 1. *Types of Agglutinogens and Agglutinins Found in Individuals of the 4 A-B-O Blood Types, and the Cross Reactions of These Bloods*

TYPE OF BLOOD	AGGLUTINOGENS PRESENT	AGGLUTININS PRESENT	AGGLUTINATION REACTION OF SERUM WITH RED BLOOD CELLS OF GROUP			
			O	A	B	AB
O.....	O	α and β	−	+	+	+
A.....	A	β	−	−	+	+
B.....	B	α	−	+	−	+
AB.....	A, B	−	−	−	−	−

In actual fact, the situation is considerably more complex than shown in Table 2, because of the occurrence of subtypes of group A, occurring either alone, as A_1, A_2, A_3, A_4 and possibly others, or in combination with B, as A_1B, A_2B, A_3B, etc. These subtypes are apparently determined by distinct forms of the "A-gene," since they breed true. The A-B-O types are thus not determined by a series of three allelomorphs, but by a series of at least six.

A tremendous amount of work has gone into the characterization of the substances responsible for the A-B-O reactions; the considerable information available has been summarized by Kabat.[17] They are mucoproteins, in which four different sugars and 11 different amino acids have been shown to be present From the genetic standpoint, here is still another illustration of how genes act through controlling chemical specificities.

THE RH SYSTEM. The tempo of discovery as regards the Rh antigens is a telling commentary on the pace of scientific research today. In 1939 and 1940 a new antigenic property of human red blood cells was defined in a series of penetrating papers by three American investigators, Landsteiner, Wiener and Levine. This property, termed, for reasons we need not go into, the Rhesus (Rh) factor, was shortly shown to be transmitted in families as if due to a dominant gene. The clinical significance

of the discovery lay in the fact that infants who had inherited this property from their fathers, but whose mothers lacked the property, might while *in utero* stimulate the formation of antibodies on the part of the mothers, with the subsequent development of hemolytic disease in the infants. The discovery thus provided the solution to a long-standing clinical enigma, hemolytic disease of the newborn.

TABLE 2. *The Various Possible Marriages with Respect to the Various A-B-O Blood Types, and the Possible Kinds of Children from Each Marriage**

	TYPE OF MATING	BLOOD GROUPS OF CHILDREN
1.	OO x OO	OO
2.	OO x AO	AO, OO
	OO x AA	AO
3.	OO x BO	OO, BO
	OO x BB	BO
4.	AO x AO	AA, AO, OO
	AA x AO	AA, AO
	AA x AA	AA
5.	AO x BO	AB, AO, BO, OO
	AA x BO	AB, AO
	AO x BB	AB, BO
	AA x BB	AB
6.	BO x BO	BB, BO, OO
	BO x BB	BB, BO
	BB x BB	BB
7.	OO x AB	AO, BO
8.	AO x AB	AA, AB, AO, BO
	AA x AB	AA, AB
9.	BO x AB	AB, BB, AO, BO
	BB x AB	AB, BB
10.	AB x AB	AA, AB, BB

* The types shown in braces cannot in practice be distinguished from one another except on the basis of the kinds of children produced.

It shortly became apparent that there were several different kinds of Rh-positivity, and that the different Rh antigens which it was necessary to postulate occurred in an overlapping rather than exclusive manner, in the sense that one individual could apparently possess several kinds of Rh antigens at once. It further became apparent that of the four antigenic properties known at that time, identified by the antiserums, anti-Rh_o, anti-rh′, anti-rh″, and anti-hr′, two of them had a reciprocal relationship, in that an individual whose cells failed to react with anti-rh′ always reacted with anti-hr′, and vice versa. Wiener interpreted these findings, taken in conjunction with the relative frequencies of the various antigens, as consistent with the hypothesis that the reactions were determined by a series of multiple alleles, each with

multiple antigenic properties. At the same time a very active group of British investigators had become interested in this field, one of whom, R. A. Fisher, suggested an alternative hypothesis, namely, that the reactions were determined by a series of three closely linked loci, with anti-rh′ and anti-hr′ identifying two genes at one locus, termed *C* and *c*, anti-Rh_0 identifying one gene at the second locus, termed *D*, and anti-rh″ identifying a gene at the third locus, termed *E*. The discovery, a few years later, of an anti-hr″ was felt by the English group to identify an allele of *E*, termed *e*. As Race and Sanger have pointed out, the findings at the lozenge locus in Drosophila provide an excellent analogy for the situation postulated for the Rh antigens. The relationship between the series of multiple alleles postulated by Wiener and the linkage

TABLE 3. *Gene Symbols Introduced for the Rh Locus by Wiener, and Their Equivalents in the Fisher-Race Scheme*

WIENER	FISHER, RACE
r	cde
r′	Cde
r″	cdE
r^y	CdE
R^o	cDe
R^1	CDe
R^2	cDE
R^z	CDE

groups postulated by the British is shown in Table 3. The technical details of how, by use of the appropriate antiserums, one establishes the presence in an individual of any one of these alleles or linkage groups is beyond the scope of this paper, as is a description of the less common Rh variants.

The past 10 years have witnessed a lively and acrimonious debate concerning the relative merits of the two theories. The problem of deciding whether the Rh reactions are to be regarded as due to one or several genes is complicated by the fact that the working concept of a gene is undergoing constant modification.* Three criteria enter into the definition of a gene, namely, the ability to mutate, the ability to be separated from its neighboring genes through chromosomal crossing-over, and the ability to discharge a specific function in cellular metabolism. There is urgent need for a dispassionate review which will evaluate the two conflicting theories from the standpoint of these three criteria, the more so because of the basic genetic principles involved. For those who prefer to think of the gene as the unit of chromosomal crossing-over, and there are many, the "Fisher theory" cannot be regarded as proved in the strict sense until crossing-over has been observed

* See Symposium on Pseudoallelism and the Theory of the Gene. *Amer. Nat.*, 89: 65–122, 1955.

between the supposedly separate genes involved. Because of the difficulties in working with problems involving close linkage in man, a satisfactory demonstration of crossing-over between the Rh loci may prove extremely difficult. I cannot accept the statement in the second edition of Race and Sanger's book[34] that the Fisher theory "has now been confirmed in practically every detail and is accepted universally." Furthermore, to say of the postulated genes, as these authors do, that "linkage is probably very close, perhaps even absolute," is to evade the issue of what is a gene, since, as just noted, one operational definition of a gene is that unit of heredity which cannot be subdivided through crossing-over.

But though I am unable to accept the British concept of the Rh locus as proved, there can be no doubt of the utility of their nomenclature. When a single symbol is used, as in the Wiener system, to represent a complex of serological reactions, not only are there greater demands on the memory (and greater possibilities for confusion on the part of those whose memories are less than perfect), but also each new discovery of an Rh variant logically demands the development of a new set of symbols. The British system is much more flexible in this respect.

Other Inherited Serological Systems. In addition to the two serological systems just described, no less than 18 other separate and distinct inherited antigenic systems have been identified. In essence, the genetics of these systems are simple: the production of the antigen or antigens which define the system is under genetic control; a child can produce a given antigen only if the gene responsible for the antigen is present in one or the other of its parents and transmitted to the child (barring mutation). The systems are all of clinical significance either as a cause of hemolytic disease of the newborn or of transfusion reactions. As in the A-B-O and Rh groups, hemolytic disease arises when a fetus has inherited from the father the capacity to form an antigen not present in the mother, with resulting stimulation of antibody formation in the mother. Likewise, transfusion reactions result when a person is repeatedly given blood containing an antigen not present in the recipient. Nine of these 18 additional systems are "private," a term suggested by Levine, in the sense that the antigen (or lack thereof) which identified the system because it led either to hemolytic disease or a transfusion reaction has thus far been encountered in only one or at most several families. Several of these systems are already known to have a degree of complexity approaching the Rh findings, and raise the same basic genetic questions. A more or less standardized approach to the nomenclature problem has been introduced for the more recently discovered blood groups, which is a valuable step towards minimizing confusion based on matters of terminology alone. The book by Race and Sanger[34] is an excellent summary of the present status of this

rapidly moving field; the collected papers of Wiener[46] on the Rh types should be consulted by those interested in this particular problem.

Hemoglobin Types

In 1949 Neel demonstrated that the sickle cell trait and sickle cell anemia were related as heterozygote to homozygote, i.e., that there was in Negro populations an abnormal gene which, when present in single dose, resulted in the asymptomatic sickle cell trait, but in double dose, in sickle cell anemia. Independently, in that same year, Pauling and his associates reported that the basis for the sickling phenomenon was an abnormal hemoglobin, whose presence was readily identified by the techniques of electrophoresis. In simple heterozygotes for the sickle cell gene, some 30 to 40 per cent of the hemoglobin was abnormal, whereas in homozygotes virtually all the hemoglobin was abnormal, the remainder having the characteristics of the hemoglobin usually encountered in the fetus or newborn infant. This so-called fetal hemoglobin has normally largely disappeared by the sixth postnatal month. In the eight years since that discovery no less than seven additional inherited abnormalities of human hemoglobin have come to light. Normal hemoglobin is designated hemoglobin A, fetal-type hemoglobin as hemoglobin F, the nine variants as C, D, E, G, H, I, J, K and S. An additional type of abnormal hemoglobin, that present in congenital methemoglobinemia, is designated M. The reader interested in the details of the discovery, the properties and the clinical effects of these hemoglobins will find them summarized in a number of recent review articles,[5, 14, 15, 16, 23, 24, 28, 40, 45, 47] as well as elsewhere in this volume. The presence of each of these abnormal types of hemoglobin appears to be conditioned by a single gene. The genes responsible for hemoglobin I and at least one type of M appear to behave as recessives. All the genes responsible for the other abnormal hemoglobins appear to be similar to the sickle cell gene, in that in the heterozygote they result in some 30 to 40 per cent of the hemoglobin being abnormal in type. In further agreement with the sickle cell gene, homozygosity for the genes responsible for hemoglobins C and E appears to result in 100 per cent of the hemoglobin being of the abnormal type. Thus far the results of homozygosity for the genes resulting in hemoglobins D, G, H, J and K are not known.

On *a priori* grounds there are two possible genetic relationships of the genes responsible for the abnormal hemoglobins, namely, that they are the product of a series of multiple allelomorphs, or that a number of different chromosomal loci are involved, the various genes being inherited independently of one another. Progress in elucidating these genetic relationships depends on the careful study of the families of

those rare persons in whom the genes responsible for two hemoglobin abnormalities are present simultaneously, and for this reason will be slow. The contribution which good case reports on such families make to a solution of the genetic problems can scarcely be overemphasized. Thus far it has been shown that the genes responsible for hemoglobins C and S are allelic, while the production of hemoglobin G apparently depends on abnormality at a separate chromosomal locus. The relationship of the genes responsible for the other abnormal hemoglobins to these two loci has not yet been established.

A rapidly expanding field such as this obviously calls for as elastic a system of gene terminology as possible. Such a system would seem to be provided by the following proposal:[25] The various loci associated with the production of abnormal hemoglobin can be designated by the general locus symbol *Hb*. Since two loci have already been implicated, those should be distinguished as Hb_1 and Hb_2. The precise gene occurring at the locus is indicated by a superscript. Thus at the Hb_1 locus we must distinguish between the genes responsible for hemoglobins S and C, namely Hb_1^S and Hb_1^C, as well as the normal gene, Hb_1^A, in whose presence hemoglobin A is formed. At the Hb_2 locus we recognize only the gene responsible for hemoglobin G, Hb_2^G, and its normal allele, Hb_2^A. This is simply a descriptive scheme which permits of ready modification.

Morphological Types

Perhaps the best known of the inherited morphological abnormalities of the erythrocyte is *thalassemia*, described in detail elsewhere in this volume. This defect is due to a single gene which, when heterozygous, produces a mild disorder known as thalassemia minor, and when homozygous, the much more serious disorder known as thalassemia major. There is a considerable range in the expression of both the minor and major forms of this disease, a fact which has led some workers to recognize further subdivisions of the disease, as thalassemia minima or thalassemia intermedia. In recent years it has become apparent that some of the "intermediate" forms of thalassemia are actually due to the combination of a single thalassemia gene with another gene responsible for the production of an abnormal hemoglobin. Generalizing from this—as well as the background of experimental genetics,—it seems quite likely that much of the range of expression of thalassemia minor and major can be attributed to the action of modifying genes. The use of such terms as "minima" and "intermedia," while descriptive, only serves to obscure the fundamental genetic dichotomy which exists between the two chief forms of this disease, and for this reason should be abandoned.

The nature of the basic, gene-determined defect in thalassemia is poorly understood. The finding that in children with thalassemia major virtually all the hemoglobin is fetal in type gave rise to the hypothesis that the basic defect was a block in the production of adult-type hemoglobin, the appearance of fetal hemoglobin being a compensatory mechanism.[35] From this point of view the morphological abnormalities were somehow secondary to defective hemoglobination of the erythrocyte. The more recent finding that in thalassemia major there is a marked degree of intramarrow hemolysis[8] makes it conceivable that the more important defect is one of cell structure, the most defective cells being destroyed before they are ever released into the systemic circulation. The appearance of large amounts of fetal hemoglobin in this disease would then be a secondary response to early and prolonged anoxia.

The decision as to whether or not to regard the thalassemia gene as a "hemoglobin gene" should be determined in part by the physiological and genetic relationships of this gene to those responsible for the abnormal hemoglobins. The physiological relationship is unusual in that individuals heterozygous for either the Hb_1^S or Hb_1^C genes may, when simultaneously heterozygous for the thalassemia gene, sometimes produce a much larger proportion of S or C hemoglobin than would usually be the case, but other times produce about the same amount as in the absence of a thalassemia gene. This has led to the suggestion that there may be several different thalassemia genes.[15, 45]

The genetic relationship of the thalassemia gene or genes to the Hb_1 and Hb_2 loci is at this writing uncertain. If the thalassemia gene is nonallelic to the Hb_1^C and Hb_1^S genes, individuals simultaneously heterozygous for either of these genes and the thalassemia gene should be able to produce children who are either completely normal or who possess both the genes in question. Two instances have been reported of the marriage of a person heterozygous for both the thalassemia and sickle cell genes, or the thalassemia and hemoglobin C genes, with a normal person resulting in children who either appeared to have received both the abnormal genes present in the parent, or neither,[39, 41] a finding which would seem to exclude allelism. On the other hand, Ceppellini[4] has observed that among 17 offspring from four marriages of persons simultaneously heterozygous for the thalassemia and Hb_1^S genes with normal persons, there were 10 with the sickle cell trait and seven with thalassemia minor, a finding which would ordinarily be indicative of either allelism or genetic linkage. If there is only a single chromosomal locus responsible for the thalassemia phenotype, then the data as they stand are most consistent with the hypothesis that the thalassemia and Hb_1 loci are linked, but obviously one would be happier in this conclusion if more data were available. The possibility of sev-

eral different genes producing the morphological abnormality we term thalassemia must always be kept in mind. Further observations on "critical" families are greatly to be desired.

A second inherited abnormality of the erythrocyte which by tradition should be classified as a morphological abnormality is *hereditary spherocytosis*. The discovery that there is a relative deficiency of potassium in the erythrocytes in hereditary spherocytosis,[20, 38] and that P^{32} administered in the form of $NaH_2P^{32}O_4$, although incorporated at a normal rate into the red cells, is partitioned differently in the erythrocytes of hereditary spherocytosis,[30, 31] raises the possibility that the basic defect here is enzymatic. The significance of the finding is rendered questionable by the fact that the same abnormality is observed in some instances of acquired spherocytosis, although to further complicate the picture, adenosine will reverse this finding in some but not all cases of hereditary spherocytosis, but not in acquired spherocytosis.

Hereditary spherocytosis is commonly said to be inherited as if due to a dominant gene. However, the effects of this gene when homozygous are unknown. If, as seems likely, the effects are much more marked when homozygous than when heterozygous, this would be the same type of inheritance as observed with respect to the abnormal hemoglobins and thalassemia, for which the term "incomplete or semidominance" would seem preferable.

Two other inherited morphological abnormalities which should be mentioned are *ovalocytosis* and *acanthrocytosis* (the latter a peculiar malformation of the erythrocytes characterized by protoplasmic projections of various sizes and shapes). Both are inherited as dominant traits, subject to the same reservations raised with respect to hereditary spherocytosis.

Metabolic Deficiencies

Three certainly or probably inherited abnormalities in red cell metabolic systems are known. In two instances the abnormality may, or probably does, involve other types of body cells, but has been first studied in erythrocytes. In addition, as mentioned above, hereditary spherocytosis may fall in this category.

Congenital galactosemia is a disease of many manifestations—failure to gain weight, mental defect, cataracts—all due to the inability of the body to metabolize galactose normally, and relieved by a galactose-free diet. It is known that normal erythrocytes contain a 4-enzyme system capable of converting galactose to glucose-1-phosphate. It has now been shown that in congenital galactosemia, one of these enzymes, PGal-uridyl-transferase, is lacking.[13] Whether other enzyme systems utilizing galactose are involved is not known. Although there is no doubt of

the hereditary nature of the disease, it is not yet clear whether it is due to a dominant gene with variable manifestations or, more likely, to homozygosity for a recessive gene with mild and variable manifestations in the heterozygote.[12]

Congenital porphyria is an abnormality of porphyrin metabolism characterized by photosensitivity, a hemolytic anemia with splenomegaly, the excretion of red urine and, less regularly, hypertrichosis and erythrodontia. Recent work would seem to indicate that the normoblasts of affected persons probably form excessive amounts of porphyrin which they release into the plasma in the course of cellular maturation.[37] Interestingly, not all the normoblasts seem to participate in the abnormality. The disease appears to be inherited as a recessive trait.

Almost 10 per cent of American Negroes show rather marked intravascular hemolysis following the administration of primaquine as an antimalarial. In a series of beautiful papers, Beutler, Dern, Alving and their collaborators (references in Carson et al.[3]) have shown that in *primaquine-sensitive erythrocytes* there is a deficiency of glucose-6-phosphate dehydrogenase activity; it is presumably this enzymatic deficiency which in some way is responsible for the susceptibility to primaquine-induced hemolysis. Although genetic studies have not yet been carried out on this interesting defect, it seems quite likely that it has a hereditary basis.

Miscellaneous

We are left with several ill-defined and rare defects which are probably most closely allied to the foregoing group. These include the *Fanconi syndrome* and the type of *familial hypoplastic anemia* of childhood described by Estren and Dameshek,[7] both apparently due to recessive genes; the *sex-linked, hypochromic, microcytic anemia* described by Cooley[6] and by Rundles and Falls;[36] and *familial, nonspherocytic hemolytic anemia*, the latter term undoubtedly embracing a diverse collection of entities.

THE GENETICS OF THE WHITE BLOOD CELL

It is an interesting fact, the explanation of which is by no means obvious, that despite the much greater visible complexity of the various types of leukocytes in contrast to the erythrocyte, only a relatively few inherited abnormalities of the leukocytes have been recognized. These involve for the most part either departures from the proportions in which the principal types of leukocytes normally occur or qualitative differences in the leukocytes. Among the former should be mentioned *familial neutropenia* and *familial eosinophilia*, poorly defined entities

which have been reviewed by Neel.[22] *Infantile genetic agranulocytosis* is an excessively rare but somewhat more clearly defined abnormality, characterized by a complete or almost complete lack of granulocytes in the peripheral blood. Death from overwhelming sepsis usually occurs during early infancy. The disease is inherited as if due to homozygosity for a recessive gene.[18]

The best known specific inherited abnormalities of the leukocytes are the *Pelger-Huët nuclear anomaly* and the *Alder anomaly*. The former consists in a hyposegmentation of the polymorphonuclear leukocytes, an inherited shift to the left, as it were, which is transmitted as if due to a dominant gene (review in Nachtsheim,[21] Harm[10] and Klein, Hussar and Bornstein[18]). This, again, must be regarded as a semi-dominant, since on the basis of the findings in a single child born to parents heterozygous for the gene, it seems probable that homozygous persons will exhibit virtually complete absence of any segmentation in the nuclei of the "polymorphonuclear" leukocytes. Alder's anomaly, the manner of inheritance of which is unclear, consists in an abnormal appearance in the granulation of all the principal cell types.

THE GENETICS OF THE PLATELET

The subject of inherited variations in platelets has been and remains controversial. All the recognized variations have come to light because of hemorrhagic diatheses. The possibility of differences from one person to the next which are not associated with bleeding tendencies is essentially unexplored, although the recent identification of platelet types represents a first step in this direction (literature in Blattner[1]). Platelet defects may be either quantitative or qualitative. On the quantitative side, there are a number of studies suggesting the occurrence of *essential thrombocytopenic purpura* in several members of one family. Because of the demonstration of a circulating "thrombocytopenic factor" in some persons with essential thrombocytopenia, reports of the occurrence of the disease in a mother and her newborn child cannot be taken as evidence for a genetic factor. Although this would eliminate several case reports, there are still left a number of apparently acceptable families; the mode of inheritance of the trait is not clear.

On the side of qualitative defects, the interested reader will find an excellent review in the recent article by Braunsteiner and Pakesch.[2] These authors feel that two distinct entities can be separated out of the group of coagulation defects attributed to qualitative abnormality of the platelets. The first of these, *thrombocytoasthenia*, is characterized by defective pseudopod formation and lack of "spreading" of the platelets in all plasmas and serums, although the platelets exhibit normal behavior in saline or plasma substitutes. Clot retraction is disturbed.

There would appear to be a strong overlap between this group and the patients described by Glanzmann in 1918. There is evidence in some cases of inheritance as a recessive trait. The second of these, *thrombocytopathia*, is characterized by platelets which exhibit normal pseudopod formation, but fail to agglutinate normally. The platelets are often "giant." Clot retraction is normal, but a thromboplastin generation test of the platelets is grossly disturbed. Again there is some evidence for a recessive type of inheritance. These two entities constituted between them about half of a group of 23 cases with presumptive qualitative platelet diseases studied by Braunsteiner and Pakesch. With the techniques now available it seems likely that this group of hemorrhagic diseases will undergo further clarification in the near future.

THE GENETICS OF CERTAIN SERUM PROTEINS

In this section we shall restrict our treatment to those inherited variations in serum proteins which are associated with coagulation defects, although, had space permitted, certain other inherited variations in serum proteins would have been reviewed also. The tempo of discovery as regards coagulation defects and the genetics thereof has been no less striking than with respect to the blood groups and hemoglobins, but the "outsider" attempting to review this field is confronted by a confusion in terminology and conflict of opinion which renders his work difficult. Fortunately, there have been two excellent recent reviews;[9, 43] we shall draw especially heavily on that by Graham.[9]

In any discussion of the genetic control of the coagulation mechanism it is necessary to adopt some schema of the sequence of events in blood coagulation. The wide differences of opinion among investigators of this phenomenon give a reviewer a considerable range of choice. For our purposes we shall adopt the schema presented by Graham[9] shown in Figure 4, which has the indubitable advantage of being less specific than many others and so less apt to be quickly outmoded. According to this and most other schemas, the two final reactions in coagulation are, first, the conversion of prothrombin to thrombin, and then, under the influence of thrombin, the conversion of fibrinogen to fibrin. Beyond this, at least nine "convertibility factors" have been described as contributing to the "thromboplastic effect" which, in the presence of ionized calcium, is responsible for the conversion of prothrombin to thrombin. It is with respect to the relation of these factors to one another, and to the prothrombin-thrombin conversion, that most of the current disagreement among coagulationists arises. A review of the evidence for the existence of each of these "convertibility factors," as well as a characterization of the respective factors, is beyond the scope of this review. The factors appear to occur in the blood in only

trace amounts, a fact which contributes greatly to the difficulties in their characterization.

Evidence has been brought forward for the genetic control of the formation of no less than nine of the components of the coagulation reaction given in Figure 4. The situation with respect to platelets has just been briefly reviewed and will not be discussed again. The evidence as regards the genetic control of three of the remaining factors is rather sketchy and will not be considered in any detail. These are PTA (plasma thromboplastin antecedent), the "fourth thromboplastin com-

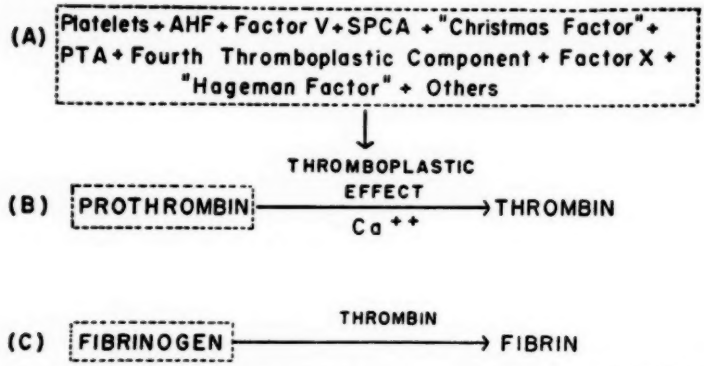

Fig. 4. A diagram illustrating the sequence of events in blood coagulation. The order in which the various factors shown in the box labeled A combine to exert the "thromboplastic effect" is unknown. Several parallel reactions may be involved. (By permission from Biochemical Genetics of Blood Coagulation, by J. B. Graham. *Am. J. Human Genet.*, Vol. 8.)

ponent," and the "Hageman factor." There are left for discussion five factors and the diseases corresponding to a deficiency of each.

The first and best known of these is antihemophilic globulin (AHF) deficiency, or *hemophilia,* recognized for many years to be inherited as a sex-linked trait. The disease varies considerably in its severity from one affected person to the next. Brinkhaus and his collaborators have presented highly suggestive evidence that these variations in severity may be due to multiple forms (alleles) of the hemophilia gene, since the severity of the disease is more or less constant in a family. A seeming paradox is the fact that (heterozygous) female carriers of the gene responsible for a mild form of hemophilia can frequently with suitable tests be shown to have a slight and subclinical coagulation defect, whereas carriers of the gene responsible for the more severe form seldom if ever are found to have even slight defects.

The second of the five factors to be discussed is the "plasma thromboplastin component" (PTC) or *Christmas* factor, the latter term based

on the name of one of the first families in which its absence was recognized. Like hemophilia, this disease is inherited as a sex-linked recessive trait and, indeed, until the recognition of the factor in 1952, patients deficient for the factor were grouped with hemophiliacs. It now appears that approximately 16 per cent of all persons who would previously have been considered to have hemophilia actually have Christmas disease. It will be a matter of great genetic interest to determine how closely the genes responsible for these two diseases are linked. Since this information depends either on the study of families in which both diseases occur, or on working out their spatial relations with other sex-linked genes, it will be some time before data are at hand. A theoretical possibility which immediately presents itself is that the two traits are due either to immediately adjacent genes or to "pseudoalleles," the former being the type of relationship discussed with reference to the Rh factors. Two sex-linked forms of diabetes insipidus and color blindness are also known; the same theoretical questions arise concerning their spatial relations.

Factor V (labile factor, accelerator globulin) is the third component of the coagulation reaction which is rather clearly under genetic control. This is a relatively labile factor, a deficiency of which produces a bleeding tendency often termed *parahemophilia* because of its similarity to hemophilia. There is suggestive evidence that the disease is due to homozygosity for a recessive gene and, in at least one large family, evidence that persons heterozygous for the gene may have a subclinical deficiency of factor V.

"Serum prothrombin conversion accelerator" (SPCA), otherwise known as "proconvertin," "factor VII," or "stable factor," is, on the basis of limited family studies, very likely under genetic control, but the details are still obscure.[27] It is noteworthy that if serum prothrombin conversion accelerator is an altered form of prothrombin, as suggested by Seegers and his associates, then this is evidence for the genetic control of prothrombin, which substance, aside from the one report by Quick and associates, has not been shown thus far to be genetically determined.

Finally, we come to *afibrinogenemia*. In contrast to the other blood clotting substances just discussed, fibrinogen occurs in the blood in appreciable amounts, 300 to 500 mg. per 100 ml. A congenital deficiency of this substance appears to be determined by homozygosity for a single recessive gene. As in the genetically similar case of factor V deficiency, there is evidence that some heterozygotes for the gene may show a minor degree of fibrinogen deficiency, although much remains to be done to clarify this point.

A number of persons have been described who appeared to lack two of these coagulation factors. While this is, of course, theoretically pos-

sible, in view of the low frequency of these defects such persons should be rare. If such cases are described with a disproportionately high frequency, it raises questions either of technique or the genetic independence of these entities.

In concluding his review of this rapidly burgeoning field, Graham[9] writes that "significant genetic advances will be made rather quickly when the workers in the field appreciate the possibilities,"—a statement with which I heartily concur.

CONCLUDING REMARKS

In the foregoing brief treatment no less than 50 inherited variations in the blood cells and their surrounding medium have been mentioned. There is already fragmentary evidence for the existence of many more. In closing, I can do no better than repeat once again the thesis of the introduction, that blood is no more variable genetically than any of the other tissues of the body. But because it is so convenient to work with, it, together with cutaneous tissue, provides a model towards which our knowledge of other tissues will slowly move.

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DIAGNOSTIC PRINCIPLES IN PEDIATRIC HEMATOLOGY

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The diagnosis of nearly every blood disorder, for obvious reasons, involves the use of one or more laboratory tests. The fact that on occasion such tests require special skills, special facilities and expert interpretation by an experienced hematologist need not be discouraging to the physician who has no special training in this field. Hematology even today, in the age of specialized technical advances, is not an occult mystery reserved for the chosen few who can tell a prerubiblast from a pronormoblast under the phase microscope or who can say "Rh subzero double prime" rapidly without stuttering. Rather it is an area of medicine in which, as in any other, the majority of diagnostic and therapeutic problems are readily solved by the orderly application of basic physiological knowledge. In most cases the hematological diagnosis rests on a few relatively simple procedures—well within the scope of the average laboratory—which can be checked and interpreted by the physician in the light of the clinical findings.

DIAGNOSTIC CRITERIA

Morphology, it is true, remains a cornerstone of hematology, and one must have the proper respect for its intricacies, but must not be overawed by its difficulties any more than one must be distressed by the complexities of the coagulation factors or the subtleties of the Rh system, not to mention the hemoglobinopathies. All these facets can and must be reduced to basic principles. The increase in detailed knowledge in any one field, though at first confusing, ultimately almost always leads to a simplification of the over-all picture and thus to

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fundamental concepts which serve the physician as he deals with the patient.

The diagnosis of hematological problems rests of course by no means solely on the use of hematological laboratory procedures. The history, the physical findings, the urinalysis, in selected cases the roentgenograms, biochemical, bacteriological, endocrinological and many other sources of information must be utilized as in any other field of medicine. Hematology resembles ophthalmology in this respect, that as often as not it deals with disorders which manifest themselves first or chiefly in its special province, but are in reality only secondary expressions of systemic disturbances or of diseases affecting a distant organ. The anemias of infection, malignant disease or renal failure, the lymphocytosis of whooping cough, the eosinophilia of visceral larva migrans, the thrombopenia secondary to hepatic cirrhosis with "hypersplenism" are cases in point. It is always necessary to keep the organism as a whole in view rather than to allow the field of vision to narrow down prematurely to an area of conspicuous pathology.

It goes without saying that even in strictly hematological conditions the general background information is often of considerable importance and may lead to a diagnosis or at least a probability diagnosis before the first drop of blood has been spilled. For those concerned with children, the gestational history, the maturity or prematurity of the patient at birth and, above all, the simple knowledge of the child's age are important diagnostic aids. On age alone one can often build a probability diagnosis. In a newborn, anemia is most likely the result of hemolytic disease and, if this can be ruled out, of blood loss, antenatal, natal or postnatal. The chances are great that an anemia, however severe, in an infant between the ages of nine and 18 to 24 months is merely an iron deficiency anemia, especially when the patient was a premature baby. On the other hand, an appreciable anemia without obvious cause in a child beyond the age of three must always be a cause of alarm, because in this group nutritional anemias are rare and the thought of malignant disease, especially leukemia, or of an inherited defect of the red corpuscles such as sickle cell disease, thalassemia or congenital spherocytosis immediately comes to mind. A detailed family history at this point, knowledge of the patient's racial background may go far in suggesting the diagnosis. The family history is of obvious interest in bleeding disorders. So is the type of bleeding manifestations which one elicits in the history or observes on physical examination. Almost on this point alone one can make a tentative distinction between hemophilia, characterized by its massive ecchymotic bleeding, large spreading hematomas, involvement of joints and muscles, and thrombopenic states in which purpuric and petechial bleeding is typical.

To give one more example of this type: Marked splenic enlargement in the absence of anemia in an apparently well child suggests at once the possibility of Gaucher's disease and is an indication for bone marrow aspiration and a skeletal survey with roentgenograms, to establish or eliminate this diagnosis, though of course eventually many other conditions may have to be considered.

Blood Values

Knowledge of normal blood values, or rather of the variations occurring in the blood values of the healthy child at different ages, is of course indispensable to any physician dealing with children. A hemoglobin level of 12 gm. is normal for a one year old child, but significantly abnormal in a one day old infant. The macrocytosis and reticulocytosis of the newborn, the physiological anemia of the young infant, the lymphocytosis of early life, the hypoprothrombinemia of the neonatal period—these and other constants of pediatric physiology must form the background against which a child is judged much as height and weight tables form a basis for judging growth and development of other somatic characteristics. And one needs a general concept of the changes in blood volume and in total red cell mass and body hemoglobin which accompany the rapid growth of the first 12 to 18 months, the requirements in terms of iron which are incidental to this process, and the stresses to which the hematopoietic system is exposed in early life. Unlike the adult who, barring blood losses, needs to manufacture new red corpuscles only to the extent of replacing physiological breakdown at the rate of about 1 per cent a day, the infant, like Alice in Wonderland, must run as fast as he can merely to stay in the same place. In other words, the infantile organism must keep the hemoglobin concentration per unit of volume approximately constant while tripling the total blood volume in the first year of life.

DIFFERENTIAL DIAGNOSIS OF ANEMIAS

Much the commonest hematological problem in the pediatric age group is anemia. The etiological factors of anemia are legion. Treatment is successful only to the extent that it attacks the underlying cause. So-called shotgun therapy is often successful because it happens to include ingredients specifically required, for example, iron when the anemia is of the iron deficiency type. But this effect is nonetheless specific, and it is neither medically nor economically sound to give drugs or other substances not required. The more serious danger of failing to make an etiological diagnosis and treat the patient accordingly lies in the delay of proper treatment and the failure of therapeutic measures

taken haphazardly. The effects of antianemic therapy are, as a rule, of startlingly strict specificity. Iron deficiency anemia will respond to iron, usually in a dramatic manner, but no other anemia will. The notorious "liver and iron shots" are probably the most useless form of therapy in pediatrics, because few children have a deficiency in hematopoietic factors supplied by liver which alone will respond to any type of liver extract, while on the other hand the amount of iron supplied in this form is negligible. Splenectomy, to give another example, is almost always successful in congenital (hereditary) spherocytosis, but as a rule accomplishes little in nonspherocytic hemolytic anemias. Vitamin K preparations, properly used, are of value when a bleeding tendency is due to hypoprothrombinemia. They are completely useless to a child with hemophilia or thrombopenic purpura and, when given by injection, may actually cause harm.

There is no need to belabor the point any further. The more our knowledge increases and the more therapeutic agents become available, the more accurate and specific must be our diagnosis. But here, too, the problem is to some extent an *embarras de richesse*. A multitude of diagnostic procedures are available and often essential to the case at hand. How should one apply them, when are they indicated, and when not? In the not too distant past the blood count and the examination of the stained smear were almost the only weapons in the hematological armamentarium. Today we have the Coombs test, the bone marrow aspiration, electrophoresis, serum iron and copper determinations, cold agglutinins and warm agglutinins, the Schilling test and the Schumm test, tagging of red corpuscles with radioactive chromium or iron, alkali denaturation of hemoglobin, mechanical fragility of erythrocytes and a host of other procedures.

Basically, it is not the improvement in diagnostic techniques which counts so much as the increased understanding of abnormal mechanisms which enables us to apply them judiciously and on occasion. The majority of cases require no more than a few simple tests which, logically applied, will lead to, or confirm, an accurate diagnosis. Figure 5 is a somewhat desperate attempt to represent the logic of diagnostic progression in graphic form. It looks more complicated than it really is, and if the reader will not become immediately discouraged by the multiplicity of names he will find a rather simple dichotomy of diagnostic possibilities at nearly every step.

The diagram is based on the fact that in the last analysis every anemia results from a disturbance in the normal equilibrium $\frac{\text{Production}}{\text{Destruction}}$ + loss of red corpuscles and hemoglobin. Either production is decreased or destruction and loss are increased. Of course the two factors are sometimes combined in actual practice, but it is

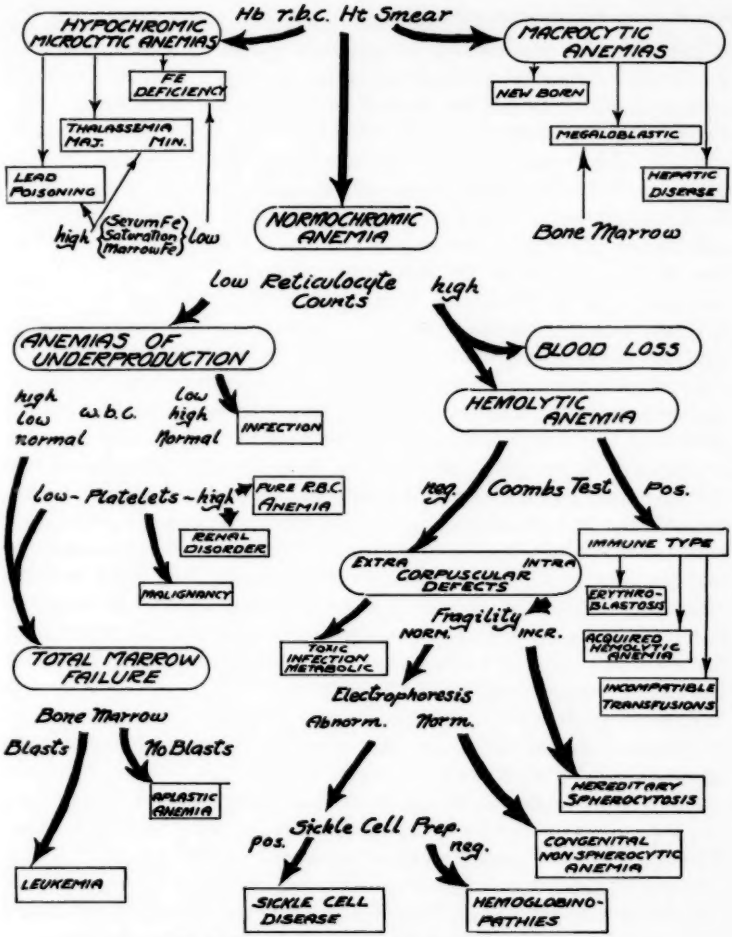

Fig. 5. Steps in diagnosis of pediatric anemias.

usually possible to determine the essential process and classify the anemia accordingly, as one of underproduction or increased destruction. (Blood loss is a relatively uncommon event in pediatrics, and usually its source is fairly obvious so that it rarely creates a diagnostic problem.) Only one other consideration is needed at this point. One must distinguish between the production or underproduction of red corpuscles and that of hemoglobin as such. When hemoglobin synthesis is lagging, whether because of lack of iron or for other reasons, microcytosis and hypochromasia result.

Hypochromic Microcytic Anemias

Step 1 in the diagnosis of anemia is as simple as it is important: the determination of hemoglobin concentration, red cell count, if possible hematocrit, and the examination of the red corpuscles in the stained film. These measures, which are available in any office or laboratory, establish at once a most important fact, namely, whether one is dealing with a hypochromic microcytic anemia or not. This permits the diagnosis of most anemias in the pediatric age group without further ado. Nearly all truly hypochromic microcytic anemias of infancy are *iron deficiency anemias.* The only other conditions one has to consider for practical purposes are rare, namely, the thalassemias and the anemia of lead poisoning. *Thalassemia major* (Cooley's anemia) rarely presents any difficulties because of the physical findings, Mongoloid facies, marked splenomegaly, grayish tint of the skin, and the great distortion in size and shape of the erythrocytes, target cells, normoblasts which are usually present. *Thalassemia minor* is more of a problem because it can resemble iron deficiency anemia. The therapeutic trial may lead to a decision, but tests are now available which usually suffice to distinguish the two conditions. In iron deficiency anemia the serum iron level is low and the iron-binding capacity high, resulting in low saturation. In thalassemia minor the iron level should be normal and the saturation high. *Lead poisoning* usually betrays itself in children by encephalopathy, lead lines in the rapidly growing long bones and by a history of pica. The anemia is rarely severe. Basophilic stippling, though not pathognomonic, can always be found.

Thus in establishing the hypochromic microcytic character of an anemia one eliminates at once a great many diagnostic problems. A word of warning is in order:

Color index or determination of the mean corpuscular constants and the appearance of the erythrocytes on smear should check each other. Too often medical technicians and physicians are misled by shrinkage artifacts on dried blood films into reporting the appearance of the red corpuscles as hypochromic. Whether they are or not should be evident from an accurate red cell count and hematocrit. The microhematocrit method of Guest and Winge is an excellent one and avoids the chore of a venipuncture.

Macrocytic Anemias

With the hypochromic microcytic anemias out of the way, one can as a rule quickly eliminate by the same method another group, those characterized by macrocytosis. It is well to remember that in the newborn period the red cells are normally macrocytic and that anemias of this age group are nearly always to some extent macrocytic anemias

by virtue of this fact. Later on macrocytosis becomes decidedly rare, except in so far as young erythrocytes, i.e., reticulocytes, tend to be larger than mature cells—hence active regeneration is associated with some degree of macrocytosis, but this will be evident by the degree of polychromasia and reticulocytosis. Otherwise macrocytosis suggests megaloblastic anemia or hepatic disease. The diagnosis of *megaloblastic anemia* requires a bone marrow aspiration. Here the aid of an expert morphologist is indispensable.

Normochromic Normocytic Anemias

On size and hemoglobin content of the red corpuscles alone one can thus go far in establishing the diagnosis of an anemia in a pediatric patient. When one turns now to the residue of normochromic and usually normocytic anemias, the great divide is the reticulocyte count, another measure well within the scope of any laboratory. The reticulocyte count indicates the extent of regenerative activity of the marrow organ. Anemia is itself a proper stimulus for increased regeneration, and if the marrow responds adequately, reticulocytosis will be present. Lack of reticulocytosis in the presence of anemia thus classifies the condition by and large as one of marrow failure, as due to underproduction of erythrocytes for one reason or another. Reticulocytosis, conversely, indicates by and large that the marrow is responding to a stimulus and therefore that the cause of the anemia is not inadequate marrow function, but either blood loss or abnormally increased blood destruction.

At step 2—these are, of course logical, not necessarily chronological steps—the reticulocyte count is therefore usually decisive in determining further diagnostic procedures, depending on whether one is dealing with an anemia of underproduction or with a hemolytic state.

Hemolytic Anemias

The hemolytic anemias in turn can be divided into immune hemolytic anemias and all others—allowing for occasional combinations and mutual complications—by the use of proper serologic methods, particularly the Coombs test. A positive Coombs test establishes the diagnosis of *hemolytic disease in the newborn* period. It usually is sufficient to make the diagnosis of acquired hemolytic anemia in older infants and children (which is of course an incomplete diagnosis until a thorough search for hidden causes such as lymphomas has been made). Rarely, a positive direct Coombs test in repeatedly transfused children indicates improperly matched blood.

With a negative Coombs test the investigation of a hemolytic process takes a new turn. One now has to decide whether one is dealing with

a congenital defect of the erythrocytes or not. Shape anomalies of the red cells observed in wet or dry preparations are of course of great help at this point. The fragility test becomes another point of dichotomy. Increased fragility usually means *congenital spherocytosis*. If at all possible, this diagnosis should be supported by a study of the parents and other members of the family.

The fragility being normal or actually decreased, one may now consider other shape anomalies and other hereditary disorders of the erythrocyte. Here sickle cell preparations and the now readily available procedure of hemoglobin electrophoresis become decisive elements of the diagnosis. A positive sickle cell preparation and a pattern showing a predominance of hemoglobin S indicate *sickle cell disease* in one of its variants. Hemoglobin C, D, E, G and the host of newly discovered combinations and permutations of hemoglobinopathies require electrophoretic analysis. The diagnosis of any obscure hemolytic anemia nowadays is incomplete without electrophoretic studies.

When all these procedures have failed to disclose the nature of a hemolytic anemia, one is left with an uncomfortable residue which may include congenital defects not apparent in morphological and chemical anomalies now measurable, acquired toxic, infectious and metabolic conditions and that vague group called "hypersplenism." History, physical findings, clinical judgment may decide the correct diagnosis in this situation, but often the help of the specialist is required at this point—and all too often it may fail. Empirical solutions, therapeutic trials are then justified, but only after an adequate study has been made. Fortunately the group in which this is necessary is steadily shrinking.

Anemias of Underproduction

On the opposite side of the great divide represented by the reticulocyte count our diagram descends to the anemias generally characterized by inadequate production of erythrocytes. This group is in itself no less heterogeneous than are the hemolytic anemias. The etiological factors lie perhaps more frequently in nonhematological conditions or at least states which are not primarily diseases of the bone marrow organ, but depress its activity. The diagnosis relies strongly on recognition of this fact, chronic infection being probably the commonest cause of such depressed marrow function.

Apart from the general clinical information and the pertinent laboratory and x-ray findings, what specific hematological criteria are helpful in this group? Knowledge of the behavior of the leukocytes is of course essential and in practice will always have been obtained at the

outset, but, as the diagram indicates, the total white blood cell count as a mere quantitative measurement is of rather limited value. It may be elevated, depressed or in the normal range in leukemia. Rarely in the acute leukemias prevailing in childhood is the number of leukocytes so greatly increased as to be in itself pathognomonic or virtually pathognomonic. Enormous increases in the total white cell count occasionally may accompany acute infections. But infection, too, may fail to produce leukocytosis and may even be the cause of more or less profound leukopenias.

More informative is, of course, the behavior of the granulocytes and the qualitative picture of the distribution of the leukocytes given by the differential count. The tremendous leukocytosis sometimes encountered in certain acute infections of infants and young children, notably pneumonia, at once betrays its benign character by the predominance of mature or nearly mature granulocytes. The equally marked lymphocytosis associated with certain other infections such as whooping cough and infectious lymphocytosis ordinarily is accompanied by only a relative granulopenia, but not by a true depression of the polymorphonuclear leukocytes. In the acute leukemias with hyperleukocytosis, on the other hand, not only is there usually a more marked degree of anemia, but also the granulocytes are absolutely depressed.

The truly leukemic forms of *acute leukemia* are as a rule readily recognized by the abnormal, primitive character of the predominant cell type, the "blast" forms, but here an expert morphologist is required. No one should make the diagnosis of leukemia on total white cell count or differential count alone, nor even on a combination of these data with anemia and the clinical picture, unless he is an experienced hematologist.

A significant laboratory finding is the presence or absence of *thrombopenia* in combination with anemia. It is often more helpful than the white cell count to know whether the platelets are adequate or decreased. Relatively few infections produce thrombopenia. It is true that thrombopenic purpura is common, but in this condition anemia is usually absent or slight unless accounted for by severe hemorrhage. An adequate platelet count in the presence of anemia is at once a fairly reassuring sign, indicating that the depression of marrow function is limited to erythropoiesis. By the same token the combination of anemia and thrombopenia is always an alarming finding suggestive of total marrow failure, regardless of the height of the white blood cell count, and therefore of such serious possibilities as acute leukemia or aplastic anemia. Unless the blood picture is otherwise unequivocally diagnostic of leukemia, a bone marrow aspiration is always indicated in this situation and will usually establish the diagnosis of leukemia at once.

DIAGNOSTIC TRENDS

It is obviously impossible to reduce all the diagnostic possibilities connected with the presence of anemia to an accurate scheme or diagram. Individual judgment and experience will always count for a great deal. Many conditions and combinations have been left out of this scheme. It was intended only to indicate the general logic of the diagnostic approach, to group together in broad categories based on physiological mechanisms the various types of anemia and to indicate the steps most likely to lead to a diagnosis. Nevertheless, as a guide for practical application such orientation can be useful. It must only be remembered that in dealing with an anemia one is dealing not with a static picture, but a dynamic process which may shift and change.

These changes, which require time and observation in some cases, may themselves be of great diagnostic importance. The diagnosis cannot always be established by a single set of findings. Sometimes it is absolutely essential to watch the development of a trend. An acute depression of erythropoiesis, a so-called aplastic crisis, may at first glance obscure the diagnosis of congenital spherocytosis or other hereditary and acquired hemolytic anemias because the expected reticulocytosis is lacking, but may appear a few days later. Variations in reticulocyte count can make it difficult to establish the diagnosis of a congenital hypoplastic (pure red cell) anemia except by prolonged observation. A toxic type of aplastic anemia may be distinguished at times by its course from an idiopathic one. Leukemia may undergo spontaneous remission which, if present at the time of the first hematological consultation, may make the diagnosis impossible.

Therapy, if begun before a firm diagnosis is established, can delay and sometimes permanently obliterate the diagnosis in many hematological conditions. Yet the promptest and most accurate diagnosis is not an academic point, but a matter of great practical, therapeutic, psychological and economic value. Unless confronted with an emergency, therapy, whether it be blood transfusion, hematinics, splenectomy or steroids, should be deferred until the diagnosis is made. In turn, the therapeutic trial may itself decide the diagnosis in certain cases.

The hematologist thus at times resembles the man who, in days now only nostalgically remembered, stands under the marquee of the movie theater and attempts to guess the story of the film from the lurid stills displayed in the window. Sometimes he succeeds, but often he must go inside and watch the action unfold on the screen before he knows what it is all about.

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NORMAL BLOOD VALUES IN INFANCY AND CHILDHOOD

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A definition of normal values must be approached guardedly and with full knowledge of the pitfalls awaiting anyone who attempts to use arbitrarily determined values to describe, as normal, various characteristics of infants and children (or, for that matter, subjects of any age). "Normal" may be well defined as average when observed values are distributed normally in the mathematical sense, and when variability is low: for example, birth weights of infants (Anderson et al.[2]). As for body temperature, the normal may represent an upper limit, below which physiological variation may commonly occur in healthy persons. Again, normal may signify the desired or optimal value: for example, pH of the blood, which is a value normally maintained within extremely narrow limits of variability, in which case normal and optimal might be used as synonyms. In a group of seemingly well premature infants studied at eight months of age the low values for hemoglobin in whole blood were distributed over a narrow range. The average value of 8.7 gm. of hemoglobin per 100 ml. described the group well; it could therefore be considered "normal" for an untreated group of premature infants of that age. It is obvious, however, that this is not an optimal value, in the physiological sense, for any group of eight months old infants. Such considerations indicate that values often designated as "normal" may be, but are not necessarily, optimal. Physiological and biochemical characteristics of the body are influenced by many factors,

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TABLE 4. *Blood Erythrocyte, Hematocrit, Hemoglobin and Thrombocyte Values, Birth to 12 Years*

	ERYTHROCYTES						HEMOGLOBIN				THROMBOCYTES	
	R.B.C. Count		Reticulocytes		Packed Volume Ht	Size MCV	In Whole Blood Hb		In Cells MCHC	Per Cell MCH	Count	
	Millions/mm.³		%		%	μ^3	Gm. %		Gm. %	$\gamma\gamma$	Thousand/mm.³	
AGE	Av.	Range	Av.	Range	Av.	Av.	Av.	Range	Av.	Av.	Av.	Range
At birth	5.7	4.8–7.1	4.35	2.50–6.50	56.6	106	21.5	18.0–27.0	38	38.0	227	140–290
First day	5.6	4.7–7.0			56.1	106	21.2	17.7–26.5	38	37.8		
End 1st wk.	5.3	4.5–6.4	1.12	0.10–4.50	52.7	101	19.6	16.2–25.5	37	37.2	235	150–320
End 2nd wk.	5.1	4.3–6.0	0.67	0.20–1.50	49.6	96	18.0	14.5–24.2	35	36.3	247	163–340
End 3rd wk.	4.9	4.1–6.0	0.63	0.20–1.30	46.6	93	16.6	13.2–23.0	34	35.6	267	177–367
End 4th wk.	4.7	3.9–5.9	0.73	0.10–1.00	44.6	91	15.6	12.0–21.8	33	35.0	280	185–390
End 2nd mo.	4.5	3.8–5.8	1.20	0.50–3.10	38.9	85	13.3	10.8–18.0	30	34.2	315	200–428
End 4th mo.	4.5	3.8–5.3	1.66	0.90–2.94	36.5	79	12.4	10.2–15.0	27	34.0	338	205–465
End 6th mo.	4.6	3.9–5.3	1.38	0.72–2.30	36.2	78	12.3	10.0–15.0	27	34.0	340	205–470
End 8th mo.	4.6	4.0–5.4	1.12	0.65–1.90	35.8	77	12.1	9.8–15.0	26	33.8	345	210–470
End 10th mo.	4.6	4.0–5.5	0.97	0.62–1.80	35.5	77	11.9	8.4–14.9	26	33.5	345	212–470
End 12th mo.	4.6	4.0–5.5	0.90	0.60–1.70	35.2	77	11.6	9.0–14.6	25	33.0	345	218–470

Data from E. C. Albritton[1]: *Standard Values in Blood*, p. 38, Table 38.

endogenous and exogenous, which must be recognized and rigidly specified in the interpretation of data on groups of individuals.

The circulating fluid, blood, is part of a tissue of the body named by Witts[21] the erythron, which includes not only the circulating cells and plasma, but also the hemopoietic tissues of the bone marrow and extramedullary blood-forming centers. The magnitude of the total organ and of its several components varies with age in the developing fetus, infant and child.[3, 20, 23] All parts of this organ are constantly and rapidly changing and are subject to many factors affecting its composition. The manufacture of plasma proteins, fibrinogen, globulins and albumin in the liver is as much a function of the erythron as is cell production in the hemopoietic tissues of the bone marrow, spleen and lymph nodes.

Much has been written on the characteristics, qualitative and quantitative, of various elements of the circulating blood. Volumes of data have been compiled to indicate what different hematologists conceived to be average, usual, acceptable or, according to individual convictions, normal values for number, size, shape and composition of the blood cells. Such tabulations have great descriptive value as guides for other investigators, but all who use them should recognize the likelihood of error in statistically derived concepts of normal values. Such considerations are especially pertinent to the presentation of data on erythrocytes and hemoglobin that follows.

The averages and ranges of values in Table 4 represent the consensus of a large number of hematologists. But one must bear in mind the fact that average figures such as these do not necessarily represent "good" levels, and that the commonly observed lower ranges (especially of hemoglobin) may be far from desirable levels when viewed as indices of nutrition.

RED BLOOD CELLS AND HEMOGLOBIN

Normal values for number, size and hemoglobin content of erythrocytes in early childhood must be defined first of all in relation to age. The widest ranges for all directly determined and calculated values ever observed among infants or children apparently in states of good health and nutrition occur during the first three years of life. Values commonly found at three months of age and considered "normal" might indicate serious disease at three days or three years. Even when age is specified, variability within each age group remains so high that it is almost impossible to decide upon a "normal" figure. Few children escape either the so-called physiologic anemia of early infancy or some degree of iron-deficiency anemia before the third year. We

believe that in any cross-sectional study of hematologic values the arithmetic average will usually be too low to be accepted as a normal figure and that the mode, although it might describe some age groups better, is probably still too low. In the absence of blood dyscrasia, chronic bleeding or chronic disease, the administration of iron will raise group averages to a higher level. Therefore it seems reasonable to use the terms "normal" and "optimal" synonymously and to accept values found among exceptionally favored children to describe the most desirable group behavior.

In a series of earlier papers[6, 7, 8, 10] we presented extensive data describing variability in the number, size and hemoglobin content of erythrocytes among infants and children representative of the Cincinnati community. Another paper[9] offers a definitive summary with statistical analyses of the data assembled from 1735 samples of blood from infants and children at ages from birth to 16 years. The group included infants of full term and premature birth and single and multiple births; many were followed individually with serial examinations of their blood from birth continuously throughout the critical years of greatest change in the hematologic values. In those earlier papers, patterns of changes were demonstrated in scatter diagrams, histograms and charts of individual records, all emphasizing the age periods in which microcytosis and hypochromia of the red cells are most likely to develop.

Table 5 presents four typical examples of the serial studies on individual infants. These data show, better than figures for group averages, the sequence of changes that must be evaluated periodically when dealing with individual infants. These four infants were in good health, consistently free from even minor illness, and showed normal growth and development. None received iron as a supplement to the natural diet. The first, S. H., maintained high values for all indices throughout four years and longer. In the second, D. Z., the values followed almost the same course except that at one year the hemoglobin in whole blood failed to increase in proportion to the red cell counts and the MCHC and MCH indices fell to moderately low levels. In the third, J. G., while the cell size, MCV, remained high, at 80 cubic microns or more, the hemoglobin in whole blood fell to 10 gm. per 100 ml. at six months of age, with MCHC 31.7 gm. per 100 ml., then rose to values like those maintained by infant S. H. throughout the next five years and longer.

Data for the fourth infant, I. R., demonstrate the progressive development of fairly severe degrees of microcytosis and hypochromia (signs of iron deficiency), followed by spontaneous recovery of normal values. It is important to note how, in this instance, slowly decreasing values for the cellular indices MCV, MCHC and MCH at two months to one year heralded early the development of microcytosis and hypo-

TABLE 5. *Erythrocytes and Hemoglobin in Blood of 4 Infants, from Birth through the First 5 Years*

		ERYTHROCYTES			HEMOGLOBIN		
INFANTS AND MOTHERS	AGE	Hematocrit Per cent	R.B.C. Millions per mm ^{3}	Size MCV μ^3	In Whole Blood Gm. per 100 ml.	In Cells MCHC Gm. per 100 ml.	Per Cell MCH $\gamma\gamma$
S.H.	Cord	42.9	4.10	105	13.1	30.5	32.0
White female	45 days	34.3	3.86	89	11.9	34.7	30.9
full term,	6 mo.	36.2	4.41	82	12.9	35.6	29.3
birth weight	1 yr.	36.9	4.98	74	12.7	34.4	25.5
3.60 kg.	18 mo.	40.3	5.19	78	13.6	33.7	26.2
Mother:	2 yr.	39.0	5.14	76	13.6	34.9	26.4
parity 1	3 yr.	38.2	4.82	79	12.8	33.5	26.6
Age 23	4 yr.	36.8	4.78	77	12.3	33.4	25.7
D.Z.	Cord	54.8	4.92	112	17.2	31.3	34.9
White male	45 da.	36.8	3.87	95	12.2	33.1	31.5
full term,	6 mo.	34.5	4.54	76	11.2	32.6	24.7
birth weight	1 yr.	39.2	5.40	73	12.4	31.7	23.0
3.28 kg.	18 mo.	38.4	5.08	76	12.4	32.3	24.4
Mother:	2 yr.	39.3	4.75	83	13.5	34.4	28.4
parity 1							
Age 35							
J.G.	Cord	59	4.80	123	19.6	33.3	40.8
White male	4 mo.	35.7	3.99	82	11.7	32.8	29.3
full term,	6 mo.	31.5	3.94	80	10.0	31.7	25.3
birth weight	1 yr.	34.5	4.23	82	12.0	34.6	28.3
4.05 kg.	2 yr.	38.5	4.61	84	12.9	33.5	28.0
Mother:	3½ yr.	37.8	4.69	81	13.1	35.0	27.9
parity 1	5 yr.	36.8	4.46	83	12.5	34.0	28.0
Age 27							
I.R.	Cord	48.4	4.02	120	15.8	32.7	39.4
White female	45 da.	44.8	3.95	113	15.3	34.0	38.6
full term,	2 mo.	35.2	3.71	95	11.3	32.0	30.4
birth weight	6 mo.	37.2	4.50	82	11.0	29.6	24.4
3.60 kg.	1 yr.	34.7	4.46	78	11.4	32.9	25.6
Mother:	18 mo.	36.4	5.72	64	10.2	28.0	17.8
parity 7	2 yr.	32.8	5.76	57	9.4	28.7	16.3
Age 42;	2½ yr.	38.7	5.18	75	12.5	32.3	24.1
interval since	3½ yr.	41.0	5.22	79	13.6	33.2	26.0
last pregnancy 6 yr.	5 yr.	38.0	4.69	81	12.6	33.2	26.9

In each, the values remained essentially unchanged during subsequent years after the last observations listed.

chromia, while the whole blood hemoglobin was maintained at levels 11.0 to 11.4 gm. per 100 ml., usually held to be satisfactory. At ages one and a half and two years the cellular indices fell to low levels, while the whole blood hemoglobin decreased to 10.2 and to 9.4 gm. per 100 ml.; but at two and a half to three and a half years all values rose to

"optimal" levels, without benefit of supplemental iron therapy, and thereafter remained high.

Our previously published serial records on individual infants demonstrated the effects of the administration of iron in infants with frank severe anemia of iron deficiency, as well as with milder degrees of anemia, leading to rising levels of hemoglobin and cellular indices similar to the pattern of spontaneous changes observed in infant I. R. More important, the serial records also demonstrated how, even among infants most likely to have iron-deficiency anemia, e.g., later siblings of anemic infants, the prophylactic administration of iron to supplement the dietary intake maintained high normocytic and normochromic values throughout the critical period when those values were most apt to fall.

Sturgeon[15] studied the influence of the administration of iron in two groups of normal infants around 14 months of age: one group with whole blood hemoglobin levels 9.1 to 10.8 gm. per 100 ml., slightly below the median for both groups; the other group with hemoglobin levels 10.8 to 13.2 gm. per 100 ml., slightly above the median for both groups. After iron therapy the mean hemoglobin levels rose slightly but significantly in both groups, respectively 1.06 and 0.61 gm. per 100 ml. The highest recorded level was 13.2 gm. Sturgeon concluded that among normal infants at this age a natural limit for hemoglobin concentration in the blood is approached in the vicinity of 12 ± 1 gm. per 100 ml., and that beyond that level the administration of iron should not be expected to lead to further increase.

Examination of many individual case histories as well as statistical analysis of grouped data suggests to us that the following figures might be accepted as most nearly approximating normal and desirable values for children during the whole age span between the neonatal period (three months) and adolescence:

Hematocrit	36.9	per cent
Red blood cell count	4.5	millions per c.mm.
Mean corpuscular volume	82.0	cubic microns
Hemoglobin in whole blood	12.5	gm. per 100 ml.
Hemoglobin in cells, MCHC	34.0	gm. per 100 ml.
Hemoglobin per cell, MCH	27.5	micromicrograms

It is obvious, of course, that the development of mild anemia with spontaneous recovery such as was observed in infant I. R. must occur often, since, among samples of blood drawn at random from large groups of infants, the incidence of microcytosis and hypochromia is high among infants one to two years old, but diminishes sharply among those of three, four and five years of age. A question that naturally arises therefore is whether such changes should be regarded as "physiological"

phenomena; if so, then it might be claimed that iron therapy is not really needed for mild degrees of anemia. Against this view, and in favor of both therapeutic and prophylactic administration of iron as routine supplementation to normal diets, are observations (1) that infants with mild anemia are apt to show a precipitous fall of hemoglobin in the whole blood to severely anemic levels during intercurrent infections; (2) that anemia appears to be associated with generally lessened resistance to infections; (3) that pica occurs commonly among anemic infants; and (4) that anemic infants often have stormy courses with various infections and may be unusually prone to suffer complications.

TABLE 6. *Correlation of Hemoglobin, and Hematocrit Values and Red Blood Cell Counts*

Hematocrit versus Hemoglobin — Red Cell Counts versus Hemoglobin

Totals	Hematocrit per cent (midpoints of groups) 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50	Hemoglobin in Whole Blood gm./100cc	Red Cell Count M/c mm (midpoints of groups) 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61	Totals
1	1	4.0	1	1
4	2 2	5.0	1 1 1	4
2	1 1	6.0	1 1	2
7	2 3 2	7.0	1 1 2 1 1 1	7
12	3 4 3 1 1	8.0	1 2 1 2 2 2	12
40	1 2 17 14 5 1	9.0	1 1 2 3 3 1 5 3 3 4 1 7 2 1 3	40
88	1 11 29 44 3	10.0	3 3 6 9 5 10 7 8 9 12 11 3 1 1	88
170	2 25 79 58 6	11.0	1 1 4 11 28 29 26 20 17 11 8 8 2 3 1	170
343	4 46 184 99 10	12.0	1 8 27 59 66 75 40 39 14 4 5 4 1	343
342	1 38 197 96 9 1	13.0	3 5 32 70 94 61 42 21 7 5 1 1	342
184	1 1 25 106 45 6	14.0	2 6 14 31 48 37 31 6 8 1	184
43	1 2 22 16 2	15.0	1 2 5 8 8 9 5 4 1	43
3	1 1 1	16.0	1 1 1	3
2	1 1	17.0	1 1	2
1241	1 0 2 2 3 9 8 33 73 177 285 328 255 176 84 30 2	← Totals →	1 0 2 1 5 5 15 38 74 143 191 239 187 157 95 40 28 12 8	1241

HIGH DEGREE OF CORRELATION r = .896 — LOW CORRELATION r = .397

The hematocrit is a more informative index of anemia than either R.B.C. count or Hemoglobin, if considered alone

Data from 1241 samples of blood from infants and children, 3 months to 14 years of age.

These are all strong arguments in favor of routine prophylactic treatment designed to keep the hemoglobin levels high throughout infancy.

It is pertinent to note here the degree of correlation between values for the hemoglobin in whole blood, red cell count and hematocrit, simultaneously determined on individual blood samples. In Table 6, data on 1241 samples of blood from infants and children from three months to 14 years of age are arranged for comparison of hemoglobin values with corresponding values for hematocrit and red cell counts. These figures were assembled as part of the unpublished statistical studies mentioned earlier.[9] The table demonstrates a high degree of positive correlation ($r = 0.896$) between the hematocrit and hemoglobin values. Far less correlation exists between the red cell counts and the

hemoglobin values ($r = 0.397$). It is obvious that high cell counts were frequently associated with low hemoglobin values. Thus the cell count, taken alone, often is not informative and may even be misleading as an index of anemia, particularly at ages when the anemia of iron deficiency is most frequently encountered.

RETICULOCYTES

In Table 4 data are cited on reticulocyte counts from birth onward. Here, again, observations on individual infants may be more informative than averages of grouped data, with regard to physiological adjustments during the first weeks of life. Washburn[17] demonstrated well the interrelated changes in postnatal adjustments of the red blood cells in individual babies, with charts depicting the progressive day-to-day or weekly changes in cell counts, hemoglobin, cell size and, especially, the percentage of reticulocytes in relation to the other changes. He observed high reticulocyte counts at birth, 4 to 6 per cent, followed in many infants by an abrupt decrease during the second to sixth or eighth week of life, while the red cell counts were falling, to around 0.5 per cent or less, then a rise of reticulocytes at eight to 10 weeks, with rising red cell counts, followed by another decrease of reticulocytes to 0.8 or 1 per cent at around four months (see his Chart 2). Decreases in red cell count, hemoglobin and hematocrit values in the first six to nine weeks of life of the normal infants seemed to be dependent on (1) a rapid increase in body mass, with increase in blood volume and consequent dilution, and (2) an abruptly decreased rate of production of red cells, indicated by the decreased reticulocyte counts. At around the sixth to tenth week the reticulocyte count rose, preceding or accompanied by a rise in red blood cell count. Washburn concluded that in most babies the rate of production of red cells between one and five or six weeks was too low for maintenance of the red cell level in the blood in the presence of an increasing plasma volume. He suggested that the stimulus to increased red cell production was the decreased red cell count and hemoglobin in the blood during the fifth to eighth week. It seems equally likely that differences in the supply of erythrocyte maturation factor, with physiological changes in gastrointestinal, hepatic and bone marrow functions, could be responsible for changes in erythropoiesis indicated by the changes in percentage of reticulocytes in the peripheral blood. Whatever may be the stimulus, cause and effect, for the concomitant changes, Washburn's final conclusion should be noted with emphasis, that the period of postnatal adjustment of the red blood cells extends over the first 15 to 20 weeks of life. At different stages of those adjustments the reticulocyte count serves as a good index of diminished or increasing red cell production.

BLOOD VOLUME

Values for blood volume in infancy and childhood have been determined by a large number of investigators, whose observations, up to 1938, were well summarized by Wollstein.[22] Compared with adults, infants were generally found to have blood volumes greater for body weight, but less for body surface.

Mollison, Veall and Cutbush[13] estimated plasma volume in infants by the dye method (T-1824) and red cell volume by using P^{32}-labelled red cells. They estimated in 28 normal newborn infants the total blood volume

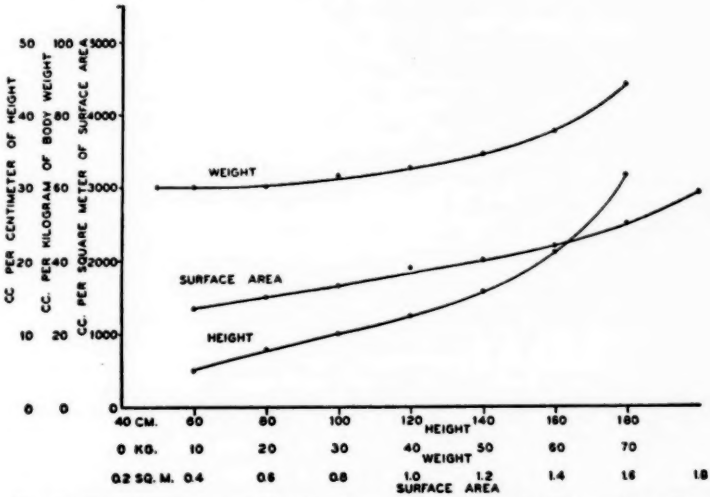
CC PER CENTIMETER OF HEIGHT
CC. PER KILOGRAM OF BODY WEIGHT
CC. PER SQUARE METER OF SURFACE AREA
WEIGHT
SURFACE AREA
HEIGHT
40 CM. 60 80 100 120 140 160 180
HEIGHT
0 KG. 10 20 30 40 50 60 70
WEIGHT
0.2 SQ. M. 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
SURFACE AREA

Fig. 6. The change in unit blood volume accompanying bodily growth. (From J. K. Brines, J. G. Gibson, 2nd, and P. Kunkel: The Blood Volume in Normal Infants and Children. *J. Pediat.*, Vol. 18.)

to be 84.7 ml. per kilogram and the total plasma volume, in 34 newborn infants, to be 41.3 ml. per kilogram. The values for total plasma volume showed roughly an inverse relation to values for the venous hematocrit: i.e., higher values for total plasma volume were found among infants with lower hematocrit values. This inverse relationship was also reported by Robinow and Hamilton.[14] The total blood volume tended to be higher when the venous hematocrit value was high. There was a elose linear relation between values for total red cell volume and those for venous hematocrit when the latter were below 55 per cent; with higher hematocrit values the total red cell volume was disproportionately higher. The ratio of the "whole body hematocrit" (total circulating red cell volume: total blood volume) to the venous hematocrit was found to be a constant, 0.87, among infants with venous

hematocrit values ranging from 17.9 to 66.2 per cent. This figure is close to that of 0.91 reported by Gibson, Peacock, Seligman and Sack.[5]

Brines, Gibson and Kunkel[4] reported measurements of plasma, red cell and total blood volumes of nine newborn infants and of 41 children at ages up to 17 years. Their data indicated that the average normal full term infant has a total blood volume of about 250 to 300 ml. and that this is doubled during the first year of life. Thereafter there is a gradual increase until the age of puberty, at which time a level of about 2500 ml. has been attained. Unit volume in terms of plasma or

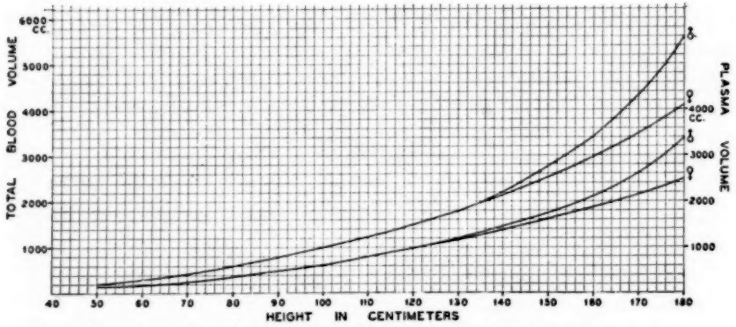

Fig. 7. Nomogram for the prediction of normal plasma and total blood volume in infants and children. The lower lines represent plasma, and the upper lines total blood volume. (From J. K. Brines, J. G. Gibson, 2nd, and P. Kunkel: The Blood Volume in Normal Infants and Children. *J. Pediat.*, Vol. 18.)

whole blood per unit of weight, height or square meter of body surface was not constant, but increased with continuing growth. For practical purposes, height appeared to be the correlative factor of choice for predicting probable blood volume. Figure 6 shows changes in unit blood volume accompanying bodily growth in height, weight and surface area of normal infants and children. Figure 7 is a nomogram for prediction of normal plasma and total blood volume from the height.

LEUKOCYTES

Many reviews dealing with leukocytes in healthy infants and children afford data indicating the wide ranges of variation in total leukocyte counts, as well as patterns of differential counts.[23] Only the general patterns of changes in the usual or average values need be defined here.

In the first 48 hours of the neonatal period the leukocyte count most often is high, but not invariably so. Counts as high as 45,000 and

as low as 3600 are recorded, with a mean figure around 22,000 per cubic millimeter. Usually the number falls abruptly by the third or fourth day to around 8000 per cubic millimeter, but during the next six months the range of variation commonly may be from 5000 to 24,000. The most frequently observed ranges in variation of leukocyte counts are approximately 10,000 to 15,000 at six months, 9000 to 14,000 at one year, and 8000 to 13,000 at two years, with an average count at two years around 11,000 per cubic millimeter. Thereafter both the variability and average figures decrease to those found among adults.

During the first six months there appears to be no evidence of an orderly rhythm in fluctuations of the leukocyte counts during the day or night, no important correlation with age, nor with activity, feedings or other events of the day. The counts done serially on individual infants tend to fluctuate within zones characteristic of each infant.

Marked variations in the differential counts are common in the first few days of life. The neonatal leukocytosis is characterized by a preponderance of neutrophils, usually around 70 per cent. With the drop in total white cell count on the third or fourth day the neutrophils decrease to about 50 per cent; from the third week to the fourth year both relative and absolute numbers of the granulocytes are less than the lymphocytes. After four years the granulocytes increase gradually, until by the ninth or tenth year the adult level of 55 to 65 per cent is reached.

The proportion of young granulocytes is elevated during the first two or three days of life, and this "shift to the left" may persist into the second week; thus the presence of immature granulocytes in the blood during this period is not necessarily helpful to diagnoses of pathological processes.

During the first two weeks of life monocytes are more numerous than at any other time in childhood and may constitute 5 to 17 per cent of the total white cells.

The limits of variation for infants two to 26 weeks of age are as follows:

CELL TYPE	PERCENTAGE	NUMBERS PER CUBIC MILLIMETER
Polymorphonuclear	5 to 63	500 to 9500
All granulocytes	10 to 68	650 to 11,500
Lymphocytes	27 to 88	2300 to 15,700
Monocytes	1 to 18	150 to 2400

Basophils seldom make up more than 0.1 to 9.5 per cent of the differential count in healthy infants and children.

The percentage of eosinophils is low on the first day of life, but then increases to 5 or 6 per cent in the next three to four days and

declines rapidly thereafter. The average eosinophile count on the first and the second days of life is, respectively, 359 and 420 per cubic millimeter, with a range from 75 to 1100,[11] figures that are much higher than in the older child and adult. By the end of the first week of life the absolute eosinophile count drops to about 275 per cubic millimeter, where it remains throughout childhood.

PLATELETS

Observations differ regarding the number and variation of platelets during infancy and childhood.[1, 3, 20, 23] The results of different investigators are actually less discrepant than one might expect in view of the fact that no method of enumerating platelets is satisfactory in every respect. Even the same method may yield somewhat different results in the hands of different observers. Because of difficulties inherent in the enumeration of platelets, the highest counts are not necessarily more correct than lower ones.

Some have found rather constant values for platelets during the first two weeks of life, while others have noted higher counts in the first two days than during the second week, and still others have reported the opposite. The majority of platelet counts have ranged from 170,000 to 400,000 per cubic millimeter during this period of life, although counts as high as 600,000 to 700,000 have been recorded.

After the newborn period and up to puberty the platelet count varies from 200,000 to 600,000 per cubic millimeter, with an average of 350,000 or more. The count may be somewhat higher during the first five years of childhood, decreasing as puberty is approached.

CONCLUSION

R. J. Williams,[18, 19] in his challenging papers dealing with metabolic patterns and biochemical individuality, insisted that attempts to define a prototype "normal man" are doomed to failure, since "this hypothetical synthetic individual simply does not exist." That view is pertinent to this presentation of selected hematologic data under the title of "normal blood values."

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ANAEMIAS IN THE NEWBORN OTHER THAN HAEMOLYTIC DISEASE

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Before haemolytic anaemia could be clearly differentiated from other anaemias of the foetus and newborn, the Rh factor had to be discovered, the hypothesis of an antigen-antibody reaction as the primary cause of the anaemia propounded and substantiated, and diagnostic methods evolved, such as the Coombs test, and the use of colloid media and of proteolytic enzymes in the demonstration of the presence of antibodies in serums.

Discovery of the Rh factor led, too, to a new flowering of our knowledge of blood groups; new systems were discovered, old ones refined. About the same time physiological and inherited variations in the haemoglobin molecule came under intensive study. The new knowledge gained in these and other fields was then used to study the apparently nonhaemolytic anaemias of the newborn; little by little this group, with which this article is concerned, is being broken down into its constituent parts and the modes of disease production made clear.

THE ANAEMIA OF HAEMORRHAGE

Most reported examples of nonhaemolytic anaemia in the baby still wet from the womb are due to acute external haemorrhage at or close to the time of delivery; they are due to the bursting, tearing or cutting of a vessel between the placenta and the baby, or on the surface or in the substance of the placenta. Some of the babies suffering this accident are in the utmost need of treatment; some can wait.

Consider first those cases in which the exact moment of bleeding is closely known, as it may be in caesarean section. Neligan and Russell's[20] excellent report gives us some valuable information. This was a study of the blood loss that may result from damage to the placental circulation when a baby is being delivered by caesarean section because of placenta praevia. In 20 of 45 cases placental tissue was found underlying the surgical incision in the lower segment of the uterus. Among the 20, they judged damage to the foetal circulation to have been slight

in one, moderate in nine, severe in one, doubtful in one, and absent in eight. In the baby in whom the damage was severe "the operator tore across what amounted to vasa praevia. About one minute elapsed between this accident and the clamping of the cord." (Note how brief the interval was.) "The baby was pale, but *did not appear ill* [italics mine]: his skin prick haemoglobin level at 30 minutes was 84 per cent (12.4 gm. per 100 ml.). An hour later he seemed rather cold and unresponsive." He died at three and a half hours. "Autopsy revealed no cause of death excepting pallor of all organs."

This case brings out several points. Although the baby was to die within three and a half hours, it did not, on first examination, appear ill, only pale. "The exsanguinated baby may appear deceptively well until shortly before death." The second point is that the baby did live for three and a half hours. I shall return to this point shortly in considering haemorrhage under somewhat different conditions. The third point is that a fall in haemoglobin was measurable within 30 minutes of birth. In another of their babies, whose placental circulation was moderately damaged, the skin prick haemoglobin was 90 per cent (13.3 gm.) at 20 minutes and 83 per cent (12.3 gm.) at 55 minutes. In Busby and Neal's case[1] the venous haemoglobin was 11.0 gm. (74 per cent) less than half an hour after massive bleeding from a small vein on the surface of the placenta; the total protein, albumin and globulin values were also down to about 75 per cent.

This question of the rate at which the haemoglobin values in a newborn baby fall after massive haemorrhage is important in diagnosis and in decision about treatment. The following case, drawn from another source, adds some information.

CASE I. A 4-pound premature baby with haemolytic disease was given a replacement transfusion of blood to which so much heparin had been added that the blood in the baby's circulation was rendered incoagulable, so that the baby bled and died. The donor blood contained 14.4 gm. of haemoglobin per 100 ml. The pertinent data are as follows:

TIME	NATURE OF SPECIMEN	BLOOD IN ML. IN-JECTED	BLOOD IN ML. RE-MOVED	HAEMOGLOBIN IN GM./100 ML.	BILIRUBIN IN MG./100 ML.	ANTIBODY TITRE IN ALBUMIN
Zero	Cord blood			2.8	4.86	++++ ++ ±
24 min.	Start transfusion	0	20	3.9	5.17	++++ ++ ±
39 min.	Transfusion	110	140	9.7	3.83	+++ ++
55 min.	Transfusion	200	230	10.43	3.34	+++ ±
69 min.	Transfusion	300	330	11.51	2.78	++ ±
84 min.	End transfusion	400	430	11.38	2.87	+
90 min.	First signs suggesting intracranial haemorrhage					
3½ hours	Skin haemorrhage					
12 hours	Intracardiac injection					
13¾ hours	Venous blood at time of death			6.51	5.22	Not done

At autopsy 6 ml. of blood were recovered from the cranial cavity from an estimated 10 ml. This blood was partially clotted. Sixty milliliters were recovered from the pericardial and left pleural cavities. This blood remained fluid; it had apparently come from the needle wound in the heart made 1 hour and 40 minutes before death. There had been oozing from the nose and mouth for some hours before death, and there were haemorrhages into the skin. The baby's blood volume would be about 200 ml., and it lost about 100 ml., in agreement with which estimate we find the haemoglobin concentration cut about in half. But at least 60 ml. of the estimated 100 ml. were lost in the hour and 40 minutes before death, so that much of the dilution probably took place in that time. The baby's haemoglobin and bilirubin curves are interesting. During the final 15 minutes during which the transfusion was being given the haemoglobin fell and the bilirubin rose. This was probably at the time the baby was bleeding intracranially, and the curves suggest that a flow of fluid into the blood took place immediately.

It is clear that, at least in massive haemorrhage, inflow of fluid resulting in blood dilution and consequent fall in haemoglobin concentration may occur with great speed in the newborn. This is contrary to the commonly held opinion, but is not out of line with the observations of Grant and Reeve[12] on acute, severe blood loss from injury in adults. Measurement of the haemoglobin shortly after an assumed haemorrhage can give information that will strengthen or weaken the assumption: if the haemorrhage has been of dangerous degree, the haemoglobin may be expected to be measurably below normal within the hour, and immediate treatment is necessary.

With this information we may turn to certain other situations in which the damage to the baby is still greater. Acute blood loss may occur while the baby is in utero and a shorter or longer period of time then lapse before its birth. We have seen that if a large vessel is severed, enough blood may be lost in one minute to kill the baby, although, in the case cited, death did not occur until three and a half hours later. This suggests, then, that, in the presence of really severe acute haemorrhage—loss of perhaps 50 per cent or more of the blood volume—we must reach and treat the baby well within three and a half hours if we are to save it. As will be indicated later, the interval is probably much less than this; true, irreversible shock may develop within an hour of the haemorrhage. A still greater haemorrhage may kill more quickly, as in the following example.

Case II. Patient (case 1) of Dr. J. P. Michaels.[19] After a sudden gush of bright red amniotic fluid during the course of labour, the foetal heart sounds became irregular and then were no longer heard. The baby was stillborn 2½ hours later, and it was found that a foetal blood vessel—it is not stated whether an artery or a vein—well out toward the periphery of the placenta had ruptured; death was due to haemorrhage, and probably occurred within minutes of the start of the bleeding.

When, during labour, bright red blood flows suddenly and in quantity from the vagina, the physician must at once prepare to treat a baby bled close unto death. It may prove that the blood was the mother's blood and the haemorrhage not immediately dangerous, but it is well

to be prepared. What an alert physician can do is well illustrated in Michael's second case and in that of Busby and Neal already referred to.

CASE III. Patient (case 2) of Dr. J. P. Michaels. The patient was admitted at 6:15 A.M. in active labour. "At 10:15 A.M. the membranes ruptured and rectal examination showed the cervix to be fully dilated. There was a sudden gush of bright red blood and the fetal heart sounds became grossly irregular. The patient was immediately prepared for delivery. Oxygen was started by mask and spontaneous delivery occurred at 10:28 A.M." The obviously bled-out baby was given a transfusion at once and made a complete recovery. The bleeding was due to rupture of a "very thin, imperfectly developed" area in the wall of the umbilical vein. I imagine the rupture of this area was due to sudden increase in intravenous pressure, and that this is true of most cases of foetal vascular rupture.

CASE IV. Patient of Drs. Busby and Neal.[1] The patient, a primipara, "was taken to the delivery room at 12 noon with the perineum bulging. The . . . membranes were ruptured artificially. A few minutes later, while a pudendal block was being given, the patient had a strong contraction followed by a gush of 50 to 100 c.c. of bright red blood from the vagina . . . Low forceps were applied immediately and [the baby] . . . delivered . . . at 12:10 p.m. [It] appeared to be suffering from asphyxia pallida, and the cord was not pulsating. Shallow respirations began spontaneously, but the child did not cry, nor did his color improve under continuous oxygen inhalation . . . the baby was suffering from an extreme degree of anemia and was in shock." Transfusion was started 10 minutes after delivery, a total of 136 ml. being given over a period of 2 hours, 27 ml. being removed for purposes of examination. The baby went home with his mother on the sixth day.

These cases illustrate several points. The first is that the physician may have little time if, by his action, he is to save the life of a baby suffering from this type of nonhaemolytic anaemia. Wickster,[30] whose writings have powerfully drawn our attention to this condition, records of his first patient, whose history was similar in essence to that of Busby and Neal's, that "while preparations were being made to give blood to the infant she suddenly died." The time interval is not stated, but it must have been short. This phrase of Wickster's recurs in other reports —"while preparations were being made the baby died." Sometimes one reads that transfusion was delayed while the whole apparatus of blood selection and cross matching was set in motion. For a newborn baby in such dire need as this, blood selection is *not* necessary. The blood that can be obtained *most quickly* is the *right* blood, blood of any group and any type, as Wickster[30] well stated. Rarely are these babies suffering from true shock at the moment of birth, although they are so described. They are suffering from acute loss of blood volume; if adequate measures are not taken quickly to bring the volume back towards normal, they will pass into a state of true and irreversible shock, perhaps within an hour. Transfusion at this latter time may lead to an appearance of improvement, but they are likely to go on to die. The anaemia in itself is not an immediate danger. The primary indication is for fluid—saline, plasma, albumin or a plasma expander such as dextran. I can find no reports of their use, although Levinson[18] pointed out the need. Personally I should be chary of dextran, a little fearful of plasma

because of the possibility of hepatitis, and would prefer saline or saline and albumin. Since the baby is to be tided over until blood is available, which it should be in less than an hour under almost the worst of circumstances, saline by itself or saline with 5 per cent glucose can be of great value; it is probably better to err on the side of too much rather than too little, and to count on giving 100 ml. at the very least. Saline preceded or followed by 20 ml. of 25 per cent human albumin—the latter given over a period of five to 10 minutes—should provide the baby with an osmotically satisfactory blood of reasonable volume with readily available protein for metabolic repair, and allow of a little greater deliberation in transfusion.

I have said that for transfusion the most quickly available blood should be used. This does not mean that the blood is to be given quickly; in fact, in my opinion, the reverse is true. Having given the first 30 ml. in perhaps five minutes, slow down and take one and a half to two hours to complete the transfusion. In these babies count on giving about 20 ml. per pound. An inadequate transfusion at this time may necessitate an additional one a few hours later.[24, 32]

Differential Diagnosis

I have reversed the usual order of presentation and placed treatment before diagnosis, for treatment must often precede certainty of diagnosis if the baby's life is to be saved. The events during delivery may make the diagnosis plain enough. As to the babies themselves, those who have suffered severe, acute blood loss are pale at birth, usually very pale. You get the impression that they are long and thin; I think this is due in part to their pallor and peripheral vascular constriction and in part to the smallness of the abdomen, which in turn probably reflects extreme constriction of splanchnic vessels. Their muscle tone is poor, but there is tone; they move a little. Most of them breathe spontaneously and may cry, but their breathing is rapid and shallow and their cry weak. The heart is rapid. The liver is not enlarged; the spleen is impalpable. The two conditions which must be differentiated from the posthaemorrhagic state following severe blood loss are asphyxia pallida and severe haemolytic anaemia.

Asphyxia pallida is the end state of severe asphyxia anoxia; it is shock, usually terminal shock. The baby is pale, often smeared with meconium, completely flaccid, unmoving, the heart slow; if the baby breathes at all, it but gasps irregularly. The baby who has severe anaemia from haemolytic disease may also be pale, he may be limp, he may not breathe spontaneously, and when he does breathe he may but gasp. Rarely is he smeared with meconium. So far the descriptions read alike, but to the discerning eye there are differences. The second

baby, the one with haemolytic disease, tends to be broad and stubby, his abdomen full. He has a slightly oedematous look, or the conformation of Cushing's syndrome in miniature. There are often haemorrhages in the skin, which frequently has a faintly yellow cast. The umbilical cord is practically always pale yellow instead of pearly white. The vessels of the cord of a baby with asphyxia pallida or severe haemolytic disease are full, but with the first the pulsations are slow and weak or impalpable, with the second fast and abnormally strong; with severe blood loss the vessels are collapsed and pulsation weak or absent. The liver and spleen are always large and readily palpable with severe haemolytic disease, but not with the other two conditions. One should be able to make one's differential diagnosis with one's eyes and fingers without resort to the laboratory, without the delay of such resort. If doubt persists, the one important laboratory test is the direct Coombs test of the baby's blood. This should not take more than 15 minutes. If it is positive, the baby has haemolytic disease. Treatment appropriate to a baby suffering from the one condition may kill a baby suffering from the other. A baby so sick with haemolytic disease as to be confused with one suffering from haemorrhage must be *bled* at once; the baby suffering from haemorrhage must receive blood or other fluids at once.

CONCEALED AND MISTAKEN FOETAL BLOOD LOSS

I have dealt so far with babies who have patently suffered severe blood loss at or just before birth. The blood is lost suddenly, in quantity, is bright red and readily seen; the bleeding is done openly. But a foetus may bleed less openly; the bleeding may be completely concealed or the baby's blood mistaken for its mother's. If blood trickling from the vagina before or early in the course of labour is bright red, think rather of foetal than of maternal bleeding, and test the blood for the presence of foetal haemoglobin. It may come from a vasa praevia or from a rupture of a foetal vessel in the placenta. So, too, an apparent excess of blood at the time of delivery of the placenta may have its source in foetal bleeding into and through the placenta, as was true in Mather's case (Case 1 of Ref. 4). I call such types of bleeding "mistaken foetal blood loss"; the foetal bleeding is not so much concealed as mistaken for something else. In true or completely concealed haemorrhage the foetal blood enters the mother's circulation; there is no external bleeding before or during labour.

So far, with the exception of one case reported by Dunsford,[6] only gross examples of these two conditions have been proved.[3, 4, 11, 12, 24] Both O'Connor[21] and Colebatch and Maddison[5] have reported cases of mild anaemia which they believed to be due to true concealed haemorrhage. Their proof depended upon the measurement of foetal

haemoglobin in the mother's blood by alkali denaturation, a method which never really measures an exact quantity. The reported increased levels seem to me so close to normal that I do not feel the proof can be considered absolute. While it is probable that bleeding of any degree may occur and hence anaemia of any degree develop, we must await finer methods before most cases can be proved. Under particular conditions such as occurred in Dunsford's case small numbers of foetal cells may be identified if one's technique is as fine and as scrupulous as Dunsford's.[7]

In all the proved cases, save Dunsford's and Shiller's, the babies have been born with severe anaemia, with a haemoglobin even as low as 3 or 4 gm. per 100 ml. In Dunsford's there was no anaemia. In Shiller's the bleeding probably took place during delivery, and the haemoglobin level in the cord blood was normal, though the baby appeared to be in shock. In the other babies reported the bleeding has probably been rather slow, so that compensation has taken place through the flow of both fluid and young red cells into the blood, and the babies are not so sick as their haemoglobin levels would suggest. They are strikingly pale and rather quiet, but they do move about and do cry. Their respirations are shallow and rapid, their heart rate fast. Their *appearance* really differs only in degree from those with acute severe blood loss. Their prognosis is quite different. The following clear example of a baby suffering from anaemia due to true concealed haemorrhage has been kindly provided me by Dr. Wolf Zuelzer.

Case V. A baby was admitted to the Children's Hospital of Michigan when it was 12 hours of age because of pallor and anaemia. It was white and disinclined to movement, but it did cry; respiration and heart rate were rapid; neither liver nor spleen was palpably enlarged. The haemoglobin was 7 gm., the bilirubin 1 mg. There were many normoblasts in the blood smear. The direct Coombs test was negative. A tentative diagnosis of anaemia due to blood loss was made. The baby was group O, Rh-positive, cDE. cde.

This was the mother's first baby. Her pregnancy had been normal. There had been no bleeding before, and no abnormal bleeding noted at, delivery. She was group A, Rh-negative, cde.cde. When the mother's blood was tested with anti-D and with anti-E, small clumps of cells were seen among the many unagglutinated cells, while no such clumps were to be seen when anti-C was used. The clumped cells were the baby's, reacting with anti-D and with anti-E. Clumping these cells out of the mother's blood by the Ashby technique reduced her red cell count by about 300,000 per milliliter. It was calculated that about 30 gm. of haemoglobin had been lost from the combined foetal and placental circulations, and that the mother's blood should contain about 5 per cent foetal haemoglobin; the amount found on analysis was 5.4 per cent. The baby was given a simple transfusion and made a prompt recovery.

Diagnosis

One is faced with a baby who is anaemic on the day of birth. There is no history of sudden haemorrhage. The presently known possibilities are haemorrhage, either true concealed or mistaken, haemolytic disease,

and so-called hypoplastic anaemia. The diagnosis may or may not be easy. If the direct Coombs test on the baby's blood is positive, you are dealing with haemolytic disease and the question is answered. The mother's blood should be obtained as soon after delivery as possible; if you recognize such a baby at birth, and you may not, even when the haemoglobin is as low as 7 gm., take a specimen from the mother at once and *save the placenta.* The mother's blood may show bilirubinemia or foetal haemoglobinemia, or both.[24] In any event have the bilirubin and foetal haemoglobin measured. Get as complete blood grouping as possible done on the baby and the mother, and if there are antigenic differences that will allow small numbers of the baby's cells mixed with the mother's to be recognized, search the mother's blood with the appropriate serums and by the appropriate techniques.[7] (The serums described by Ikin, Lehmann and Mourant[14] and by van Loghem and val Ree-van Ree[29] may prove of value here.) If the baby is group A or B and the mother group O, B or A, then not only do this but, following Dunsford, have the anti-A and anti-B agglutinins of the mother titrated. Have the reticulocytes and nucleated red cells in the baby's blood enumerated. If you are not yourself the obstetrician, ask him about any bleeding in pregnancy or during labour and about the appearance and weight of the placenta. If it has not been saved and it is not too late, rescue the placenta from the garbage can and send it to someone who knows placentas—Edith Potter in Chicago, Arthur Hertig in Boston, or Louis Hellman in Brooklyn—I suppose there are others.

At this point the diagnosis may be clear if you have found foetal cells mixed with the mother's or a gross increase in foetal haemoglobin in her blood; suggestive if you have found a hyperbilirubinemia or the absence of a normal isohaemagglutinin in the mother's blood (the latter may be due to amniotic fluid rather than foetal red cells getting into her blood) or a level of foetal haemoglobin at or just above your own normal levels. If the baby's blood contains many young cells, you can rule out hypoplastic anaemia; if it contains none or practically none, you can rule out haemorrhage.

From this point you may have to wait. If there was an increase in bilirubin in the first specimen of the mother's blood, it was probably due to destruction of incompatible foetal red cells and will fall rapidly. Under these circumstances any foetal haemoglobin in the mother's blood will disappear at least as fast, while on the other hand the maternal titre of immune antibodies will rise. If the mother has no antibodies against the foetal red cells, they will live their normal span of about 100 days, so that the measured foetal haemoglobin in the mother's blood will fall gradually—roughly 50 per cent in the first month unless the mother develops antibodies against the foetal cells in the

meantime. Antibodies against foetal red cell antigens may appear in from three weeks to three months.

Treatment

Treatment is by simple transfusion of concentrated red cells if the anaemia is severe enough to necessitate any treatment at all. Laski and Johnston[17] reported 22 cases of nonhaemolytic anaemia of the newborn seen at the Hospital for Sick Children in Toronto. The causes of the anaemia were not determined. The initial haemoglobin values varied from 5.3 to 7.3 gm., so that the cases were fairly severe. In three no treatment was given: in one of these the haemoglobin rose 3.5 gm. in two weeks. Personally I prefer not to transfuse if the haemoglobin is as high as 7 gm. and the baby is one or more days old. Smith[26] has stressed the evils of unnecessary transfusion in children, and though he dealt with chronic anaemias, my own experience with small babies leads me to believe that in them most transfusions for anaemia are unnecessary and may serve but to depress marrow functions.

POSTNATAL HAEMORRHAGE

Haemorrhage into the gastrointestinal tract or into the soft tissues may produce anaemia of appreciable degree, but never, I think, of alarming degree. The diagnosis is nearly always obvious quite quickly. Treatment for the anaemia in itself is rarely necessary.

CONGENITAL HYPOPLASTIC ANAEMIA

This disease goes by many names; for a first-class recent review, see Calvert and Robson's article.[2] So far as I can find, only one baby with this disease has been reported as having anaemia on the day of birth, namely, Kohlbry's patient;[16] on the first day it had a red cell count of 1.93 million and a haemoglobin of 52 per cent. The remainder have passed as normal for at least a week or two after birth, and sometimes longer. The first baby I ever saw with this disease had been considered normal up to the fifth week. At that time the mother, who was a highly intelligent woman, observed that the baby was pale; when it was admitted to hospital a few days later, it had a haemoglobin of 1.5 gm. and a red cell count of 500,000. It is hard to believe that the anaemia had been nearly so severe for long; one gets the impression that, at least in some cases, there is an accelerating rather than a constant rate of fall.

In most reported cases there has been no abnormal jaundice, although in one of Smith's[25] there was jaundice from the fourth day

to the third or fourth week. Pallor at a few weeks or months of age is the sign that has nearly always led to examination. At that time the babies are pale, but well nourished; in fact, their nutrition appears better than average. The spleen is not palpably enlarged; there is an anaemia, usually very marked, and nearly always normocytic and normochromic, with practically no evidence of red cell regeneration on examination of the peripheral blood and of the marrow. The other blood elements are not affected. The direct Coombs test is negative. This last point is of the utmost importance, for some babies with haemolytic disease have a persistent anaemia and little or no evidence of red cell regeneration for several weeks after birth.[10] It is also to be remembered that the bone marrow in the normal baby undergoes a period of quiescence after birth, which lasts up to about one month.[9] During this period absolute certainty of diagnosis may not be possible.

It seems probable that two or three conditions are grouped together under one name. Most of the cases have not been considered to have any haemolytic element, but one would judge from the carefully recorded haemoglobin levels in Kohlbry's case that there was a haemolytic factor in this baby.

Treatment

Up to 1953 the only method of treatment was by transfusion, which might have to be repeated as often as once in three weeks.

In 1953 Kaass and Sundal[15] reported on the use of ACTH in a four year old child. Giving the drug was followed by a reticulocytosis, a rise in red cell and haemoglobin levels and a decrease in the need for transfusion. The same year Fisher and Allen[8] reported on a child whose blood had been kept at normal levels for eight months with cortisone. Recently Sundal[28] brought the story of Kaass and Sundal's patient up to date; the tendency to anaemia has been controlled with cortisone. Calvert and Robson[2] have also recently reported relief of anaemia by cortisone in a 10 year old child whose anaemia was first described by Robson and Sweeney[22] in 1948. The response or failure of response to cortisone may serve to break this disease category down into two or more types of disease.

OTHER CAUSES OF ANAEMIA

Sepsis and Syphilis

Sepsis is now a rare cause of anaemia of the newborn. Symptoms may develop toward the end of the first week. There is usually associated a mild degree of jaundice. Diagnosis depends upon blood and urine cultures. Treatment is by antibiotics and transfusion.

Syphilis is said to produce anaemia in a newborn baby, but I have never seen it and can find no well documented record to prove that this is so.

Twin-to-Twin Transfusion

So far as I can find, a single example of this condition has been reported, one twin plethoric at birth, the other anaemic.

Anaemia of Prematurity

Although red cell and haemoglobin values fall more rapidly in the premature than in the mature baby, anaemia of any serious import rarely if ever develops in the first four weeks as the result of prematurity alone.[9] If such anaemia is present within this time, other causes than prematurity should be sought.

Haemolytic Disease of the Newborn

Before the discovery of the Rh factor and the elucidation of haemolytic disease of the newborn many babies reported as examples of anaemia of the newborn had this disease. With the Coombs test readily available and with refined serological methods the diagnosis should be rarely in doubt in the newborn period. It should, however, be remembered that the erythroblastosis of the peripheral blood is a transitory phenomenon that may be followed by a phase of extreme marrow depression. Further, unless sensitive methods are used, the Coombs test may fail in haemolytic disease due to blood group systems other than Rh and Kell. Haemolytic disease is fully discussed in other clinics in this symposium.

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THE EFFICIENT USE OF EXCHANGE TRANSFUSIONS IN THE TREATMENT OF ERYTHROBLASTOSIS

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Knowledge of the fundamental immunological problems underlying erythroblastosis fetalis has been widely disseminated. We are now aware that occasional cases arise not from maternal isosensitization to the Rh blood factor,[2] but to one of the "rare factors," and that a considerable number of mild cases are due to factors A and B.[29] Exchange transfusion is so universally accepted as the treatment of choice that some physicians use this treatment without qu. stion in all cases of erythroblastosis no matter how mild they may be.

The introduction of exchange transfusion as a treatment resulted in a decreased incidence of kernicterus and led to the concept that adequate control of hyperbilirubinemia could practically abolish this complication of the disease.[3] Later the venous pressure was found to be elevated in edematous babies. This led to the concept that such babies suffered from cardiac failure, an event which could sometimes be prevented or improved by regulating the circulating blood volume.[20]

Since the control of these two aspects has significantly lowered the mortality, the feeling has grown up in many areas that exchange transfusion is without danger and is followed uniformly by dramatic if not magical results. We feel that current practices should be examined critically in the light of accumulated experience to see whether further improvement may be made and to guard against the creeping in of erroneous practices.

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In this paper we shall not attempt to be comprehensive, nor discuss all aspects of the disease. The reader is referred to the standard textbooks of pediatrics and their reference lists for such information. Instead, we shall be concerned with the application of our current knowledge of the pathogenic physiology of this disease to improvements in the use of exchange transfusions as a form of therapy.

WHAT AN EXCHANGE TRANSFUSION WILL DO

Certain conclusions about the effectiveness of exchange transfusion may be drawn.

Desirable Effects Efficiently Accomplished

Removal of Sensitized Infant's Red Cells and Replacement with Nonsensitizable Adult Red Cells. The usual exchange using donor blood in one and a half to two times the baby's blood volume will accomplish the removal of 85 to 90 per cent of the sensitized red cells.

Regulation of the Infant's Blood Volume. The baby with severe erythroblastosis is apt to have increased blood volume, increased venous pressure, and heart failure. The exchange can be used to control the blood volume (which can be estimated by measurements of the venous pressure) and thus relieve the heart failure.

Increase in the Oxygen-Carrying Capacity of the Infant's Blood. The baby who is anemic will benefit by replacement of his blood by donor blood of a higher hematocrit. This will occur if the infant's hemoglobin concentration is lower than that of the donor blood (around 10 gm. per 100 ml. on the average).

Desirable Effects not Efficiently Accomplished

Removal of Bilirubin. So much of the infant's bilirubin lies extravascularly[9] that whole blood exchange is an inefficient method of bilirubin removal.

Removal of Antibody. Whole blood replacement is an inefficient means of removal of maternal antibody from the baby because it also is distributed widely in extravascular spaces.

Undesirable Effects Which May in Part Be Minimized by the Operator with Care and Skill

Reduction of Hemoglobin Content. The net effect of any exchange transfusion is to bring the final hemoglobin concentration of the baby to approximately that of the donor blood. This can be expected

to rise about a gram per 100 ml. in the next day as the baby eliminates the extra water and sodium which was acquired from the donor plasma by virtue of the ACD (acid-citrate-dextrose) solution.

Possibility of Infection. Infection may be introduced through contamination of blood or apparatus or through faulty technique. The usual practice of using penicillin and streptomycin as prophylactic drugs probably does not constitute the best antibiotic coverage for prophylaxis against infections today.

Adverse Effects of Citrate. The citrate of donor blood tends to lower the infant's ionized blood calcium and thus exerts a profound action on cardiac muscle. Ordinarily this effect is counteracted by the administration of calcium gluconate. Babies differ in the speed with which they metabolize the citrate and therefore in the amount of calcium they require for their safety during the exchange procedure.

Adverse Effects of Other Electrolyte Imbalances. Graham and Heyn[16] have demonstrated the infant's amazing homeostatic powers, which usually prevent gross and fatal alterations of other blood electrolytes. It is wise, however, to use relatively fresh (less than four days in the bottle) donor blood to prevent hyperkalemia. The slight edema that may form due to overadministration of sodium (present in the ACD solution) is temporary and of little consequence.

Overloading the Infant's Circulation. The operator may cause the infant harm by inadvertent or ill-advised increase in blood volume. The temptation to "leave the baby with a good hemoglobin so he won't have to be transfused again" has led to the unfortunate practice of "topping off" at the end of the exchange. A number of babies have died shortly after exchange as a result of this practice.

Exposure and Trauma. No criteria are available to assess the deleterious effects of the trauma attending the procedure of the exchange. Common sense dictates that every possible means be taken to aid the infant in preserving homeostasis. This includes measures to insure the warmth and comfort of the baby, the use of warmed donor blood, preventing of vomiting and aspiration, and the administration of oxygen to anemic babies.

For comprehensive instructions concerning the actual performance of a proper exchange transfusion, the reader is referred to several excellent monographs on the subject.[1, 5, 14] We shall discuss only those procedural details which we feel need emphasis.

PATHOGENESIS

Most workers agree that the main manifestations of erythroblastosis are due to (1) hemolysis of red cells which have been sensitized by

maternal antibody, (2) at a period of life when the mechanism for handling the products of hemolysis is barely able to prevent (physiologic) jaundice. The baby with erythroblastosis destroys his red cells in his own reticuloendothelial system because these red cells have been prepared for early destruction by coating with maternal antibody. The baby born with a fairly normal hemoglobin has been producing red cells fast enough to keep up with this excessive rate of hemolysis. This rapid rate of production is reflected in abnormal depots of extramedullary hematopoietic tissue and by the excessive number of erythroblasts in the peripheral circulation. Red cell destruction becomes increasingly noticeable after birth since the extrauterine increase in oxygen tension presumably serves to inhibit the runaway hematopoiesis.

The baby is born without jaundice, a fact which indicates that the placenta has successfully transferred most of the excess bilirubin and other heme pigments to the maternal circulation. The rapid rise in the baby's serum bilirubin and the onset of clinical jaundice after birth merely emphasize the great capacity of the placenta to perform this function. The efficiency of the bilirubin excretory function of the newborn liver is only a small fraction of that of the liver of the older child.[4] Evidence now indicates that this is probably due to an impaired ability to convert insoluble (indirect-acting) bilirubin into soluble (direct-acting) bilirubin-diglycuronate.[24]

Excessively high serum bilirubin levels are associated with damage to nerve cells, especially of the basal ganglia. Such levels rarely develop in less than two days. Injury to nerve cells may occur regardless of the cause of the jaundice. The lethargy of erythroblastotic babies has been attributed to this bilirubinemia even in the baby who does not have kernicterus. The early stages of kernicterus may be reversible to some extent if the hyperbilirubinemia can be relieved before damage becomes permanent.

A more severe example of the disease is considered an incompletely compensated hemolytic anemia in which the maximal production of red cells cannot keep pace with the hemolysis. The baby is born severely anemic and has such a handicap that he suffers cardiac decompensation and exhibits peripheral and pulmonary edema. Such babies may even be stillborn.

The foregoing concept of pathogenesis must be essentially correct. Some workers would like to explain some of the features of the disease such as occasional myocardial degeneration, hepatic necrosis or necrosis and ulceration of the esophagus and stomach upon tissue damage by antibody. Although many investigators have failed in their attempts to demonstrate tissue Rh antigen, the recent work of Brading and Walsh[8] leads to the conclusion that Rh antigen does exist in tissues other than red cells (liver, spleen, kidney, heart and brain). Furthermore, Rh

antibody can be eluted from blood-free tissues in babies dying of erythroblastosis after exchange. This does not prove that those tissues were damaged by the antibody, but it does indicate that we should still keep an open mind concerning this facet of the pathogenesis.

Figure 8 attempts to show in a schematic way the interplay of the various pathogenic factors. Here the severest cases are represented as being in hematological decompensation, and possibly because of this often in cardiac decompensation as well. The moderately severe cases

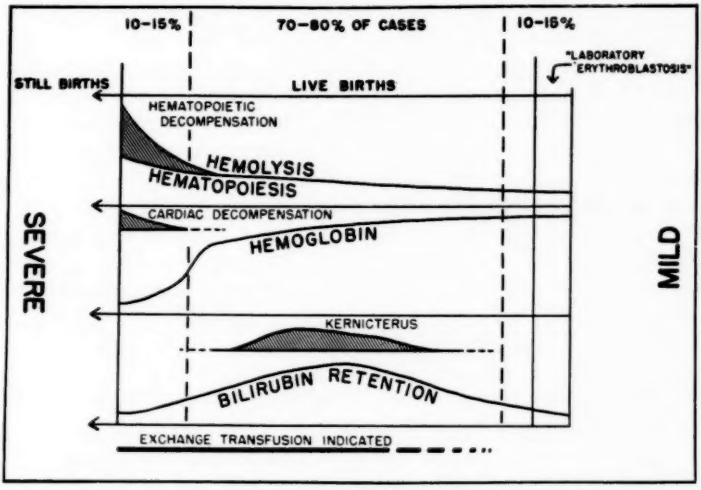

Fig. 8. Spectrum of severity in erythroblastosis (untreated).

usually have greater bilirubin retention than the very severe cases, which have less hemoglobin, or the very mild cases, which have less hemolysis.

EARLY DELIVERY FOR THE PREVENTION OF STILLBIRTHS

The question of induction of delivery before term in an attempt to lower the stillbirth rate is still not settled. The reports of Mollison[22] and Vaughn[26] showed that babies delivered prematurely had a high incidence of kernicterus and a high mortality rate. We must remember that these data were accumulated before the use of multiple exchange transfusion. Since kernicterus can practically always be prevented, the conclusions based on such data can no longer be considered valid. Evans[13] has recently reported favorably on a series of 26 cases in which preterm delivery was practiced. It is important to note that he did not deliver the baby until the clinical and x-ray findings indicated that the pregnancy was of at least eight months' duration. At present we feel

that early delivery should be attempted only if a previous pregnancy has resulted in a severely affected baby, and if the father is known to be homozygous, and if the maternal antibody is high (above 1:64). We wish to stress again that even then delivery should be delayed till there is good evidence that the baby is near term. In the past two years, our experience shows that with the early use of exchange transfusion the mortality rate is about the same for the slightly premature baby with erythroblastosis as for the full term baby.

MANAGEMENT AT BIRTH

In treatment centers such as ours, where no babies are delivered, there is often a problem of encouraging obstetricians to attend to the many little details which make for smooth management. In the past year we have furnished the obstetrician with the following "set of instructions" when we report the presence of anti-Rh antibodies in expectant mother's blood specimens we have examined for him:

1. Clamp cord at earliest safe moment—to deprive baby of excess blood volume from placenta.
2. Tie cord with 2- or 3-inch stump—to facilitate use of umbilical vein if exchange is necessary.
3. Save two tubes of cord blood (clotted): One for immediate Coombs test in your laboratory for accurate diagnosis; one to accompany baby for type, crossmatch, and van den Bergh if baby is transferred to Children's Hospital.
4. Draw specimen of mother's blood to accompany baby—for crossmatch with donor blood for baby.
5. Notify *Admitting Resident* at Children's Hospital as soon as decision is made to transfer baby—so that the laboratory and exchange team can be assembled to meet the baby on its arrival.

This effort has been eminently successful in bringing about a more decisive management of affected babies, and we are sure it has resulted in a considerable reduction of mortality in our case material of about 100 erythroblastotic babies a year.

INDICATIONS FOR EXCHANGE

If one understands the pathogenesis of the disease and the limitations of treatment, one may then construct certain indications for exchange transfusion. Nearly every worker in the field has developed his own indications. Some of these criteria are so liberal that virtually every baby with a positive Coombs test is subjected to the procedure. Others make serious attempts to select babies which would otherwise do poorly. The pediatric surgeons in our treatment center emphasize to us again and again that almost no major surgical operation which they perform, outside the neurosurgical field, carries a higher operative mortality.

If one keeps in mind the things exchange will and will not do, the

indications become clear and simple. One exchanges (1) those babies who are in cardiac failure or are so anemic that cardiac failure seems imminent, and (2) those babies who one predicts will eventually have a serum bilirubin sufficiently high to run the risk of kernicterus.

THE CIRCULATORY EXCHANGE

An exchange transfusion becomes an emergency procedure in the presence of cardiac failure. In a large series failure may be present in 5 to 15 per cent of the total cases. Detection of cardiac failure is not difficult. In mild cases the infant presents pallor and dyspnea, often with grunting respirations. The liver is large; there are rales in the lungs; and tachycardia is present. With severe failure, peripheral edema, ascites and cyanosis are also present. Such babies are usually inactive and often present ecchymoses. The most severe cases show the full-blown picture of hydrops fetalis.

The origin of the failure is not clear, but most infants with the above signs are anemic (hemoglobin below 10 gm. per 100 ml.), and many observers attribute the failure to myocardial anemia. However, this does not explain why such babies survive fetal life when oxygen tensions are low only to suffer failure within a few hours after birth when oxygen tension in the myocardium should rise. Neither do we feel that all the edema must necessarily be explained by the cardiac failure. One factor which may precipitate cardiac insufficiency at birth is the sudden load of 75 to 125 ml. of blood from the placenta which is added unless the cord is clamped promptly.

Cardiac failure may not be immediately fatal. Such babies may survive for several hours until death or relief by venesection. Much of our conviction that the above signs actually represent cardiac failure stems from the observation originally made by Mollison[20] that in such cases the venous pressure is elevated. Withdrawal of 10 to 20 per cent of blood volume (30 to 60 ml.) usually is followed by a disappearance of the rales, improvement in the color of the infant and of the blood withdrawn from the catheter, and a reduction of pulse rate. Few more gratifying maneuvers can be performed by the pediatrician.

The venous pressure is measured by holding the free end of the catheter vertically and observing the height of the column of blood above the skin surface at the umbilicus. Slight respiratory excursions should be present to show that there is an unobstructed blood column. The normal range of venous pressures in the quiet infant by this procedure is 4 to 8 cm., which should be measured accurately by means of a sterile millimeter rule. If the baby is quiet and the catheter is obviously unobstructed, pressures over 10 cm. indicate cardiac failure when accompanied by the clinical signs and symptoms mentioned previously.

The hypervolemic state may be corrected by withdrawal of blood in 10- to 20-ml. increments until the venous pressure has been reduced to 8 to 10 cm. *This venesection can be done in the delivery room, before arrangements are completed for a full-scale exchange transfusion.* Oxygenation of tissues depends upon good circulation and on hemoglobin concentration of blood rather than total body hemoglobin. Consequently, a desperately grunting, dyspneic, cyanotic baby will be greatly improved by the withdrawal of 30 to 50 ml., or in rare cases even more blood, if this restores circulatory competency. Such a venesection may be performed using a pair of sterile gloves and a sterile catheter.* As a matter of fact, the blood removed from the umbilical vein may be used for laboratory purposes. However, steps should be taken to obtain donor blood as soon thereafter as possible, since the baby will usually be severely anemic, and his cardiac function will be improved by an increase in hemoglobin concentration.

Since the survival of the infant is the immediate concern, and since exchange to the point of a respectable hemoglobin offers the baby so much, sudden availability of blood outweighs any safety measures included in elaborate cross-matching techniques.† In such grave instances the use of any O blood (preferably Rh-negative) is justified. If the blood can be brought from the laboratory with a minimum of agitation and half a minute devoted to its inversion for hanging, one can accomplish a maximum increase in hemoglobin with a minimum volume of blood. It is imperative when using such sedimented blood to exchange only that amount which will be necessary to bring the infant's hematocrit to 40 per cent or a hemoglobin of about 12 gm. This must be determined during the procedure by using a runner to the laboratory, and in our experience can usually be accomplished with 100 to 150 ml., or one may carry out Wiener's[28] practice of removing part (100 to 150 ml.) of the plasma from the donor bottle.

A further deficit should be produced during or at the end of the

* Disposable polyethylene catheters sterilized in ethylene oxide gas have recently been made available through commercial channels. This is an important innovation, since the current practice of disinfecting polyethylene materials in quarternary ammonium detergents leaves much to be desired from a bacteriological standpoint. (Mead Johnson Company., Evansville, Ind., 16 Ga. Cut Down Infusion Set, List No. 587.)

† Except in the extreme emergency described above, information obtained from the serologic laboratory should include in all cases: (1) Direct Coombs test of baby's red blood cells. (2) OAB group and Rh subtypes of mother's, baby's and prospective donor's red blood cells. (3) Crossmatch of donor's cells and mother's serum by the indirect Coombs technique. This step is necessary before transfusion except in extreme emergency (*a*) to detect the unusual instance of sensitization of the mother to "rare" antigens such as Kell, Duffy, Kidd, and others, ordinarily not determined in the donor blood; and (*b*) to detect the unusual donor blood initially classed as Rh-negative by the blood bank, but possessing the D^u antigen. The "minor" crossmatch—baby's cells and donor serum—may be omitted, since the baby's sensitized cells may autoagglutinate in any adult serum. (4) Specificity of maternal antibody. (This may be reported later.)

procedure if the venous pressure rises above 10 cm. We feel strongly that a full exchange of 500 or 600 ml. of whole blood is unwarranted for an infant as ill as we have described. If one can win a skirmish in the battle of the heart, one can fight the battle of the bilirubin later. Anticipating further procedures to regulate venous pressures or bilirubin, the operator may be tempted to tie the catheter tightly in place, fill with heparinized saline, insert a sterile syringe in the free end of the catheter, and bury the apparatus in a stack of dry sterile gauze in anticipation of reuse in a few hours. SUCH A FOREIGN BODY IS AN OBVIOUS INVITATION TO INFECTION, and scrupulous asepsis *must have been observed*. The catheter should remain in place only a few hours. In our treatment center we have found that it is actually easier to re-enter the umbilical vein with a fresh catheter than to guarantee adequate asepsis in such a complicated situation, so that we have abandoned this procedure.

A clear airway must be established and maintained. Administration of high (80 to 100 per cent) oxygen concentrations temporarily by mask or by oxyhood is beneficial in such instances of anemic anoxia.

When cardiac failure is moderate and venesection does not present the extreme emergency described above, the procedure can be more deliberate and additional precautions may be taken. The blood may be warmed in a 37° C. (no warmer) water bath while the infant is being prepared for the operation. If facilities are available for monitoring the electrocardiogram, time may profitably be taken to utilize this facility. An important safeguard in the procedure is the appointment of someone, preferably a doctor or an anesthetist, as "vice president in charge of the baby." It is the "veep's" sole job to administer oxygen, to listen to the heart tones through a flat stethoscope strapped to the left posterior chest wall, to insert a gastric catheter and remove gastric contents before the procedure is started, to use pharyngeal suction if necessary, to keep track of the increments of blood going into and out from the baby, to remind the operator when calcium is due, and to listen carefully for abnormal bradycardia during calcium administration.

It is our impression that babies in cardiac failure are more vulnerable to the calcium-lowering effect of citrate than the usual infant. This may be because the impaired circulation to the liver compromises the metabolism of citrate, allowing accumulation of this substance during the exchange procedure. In any case calcium gluconate should be administered in doses of 1.5 to 2 cc. of 10 per cent solution slowly (three to five minutes for administration) at every 100-ml. replacement, and oftener should the baby have shivering movements, irritability or retching.

Twenty-milliliter increments of blood in and out may be used in exchanging babies in good condition, and such increments are theo-

retically superior in achieving the most efficient exchange. We prefer 10-ml. increments in babies with cardiac failure.

Digitalis has been advocated in the treatment of cardiac failure in erythroblastosis, and we have used it on a number of occasions in various ways. Theoretically, it should be of some help in restoring compensation. However, if digitalis is given before exchange, one cannot accurately compensate for such variables as the fluctuating calcium, potassium and citrate levels, for the time necessary for the glycoside to become fixed to cardiac muscle, and the amount lost from the circulation during exchange. Certainly the relief of decompensation following adjustment of blood volume is so definite and predictable that this should be accomplished first without waiting for any digitalis effect. In our hands, digitalis given after the exchange has not been of obvious benefit, and it has on one occasion been accompanied by serious toxicity. We do not advise its use.

THE BILIRUBIN EXCHANGE

The distribution of bilirubin and other heme pigments within the body is only partly understood. We know that indirect bilirubin in the plasma is adsorbed to albumin. It diffuses readily from the plasma to the extravascular space, where it is probably also adsorbed to albumin. Gitlin[15] has shown that there is at least half as much albumin in the extravascular space as in plasma. One would expect the "bilirubin space" to be at least as large as this albumin space. We also know that bilirubin penetrates nerve cells. How much of the bilirubin space is accounted for by intracellular bilirubin (probably adsorbed to intracellular protein) is an enigma at the moment, but it may be large.

The amount of potential bilirubin contained in the red cells of a baby is large. A 3-kg. infant has a blood volume of about 300 ml. If he is born with a hemoglobin concentration of 17 gm. per 100 ml., he has 51 gm. of hemoglobin. If hemolysis lowers this concentration to 7 gm. per 100 ml., 30 gm. of this hemoglobin will be metabolized to produce about 1050 mg. of bilirubin. If we were to assume (incorrectly) that none of this bilirubin was excreted, and that it was equally distributed throughout all the body water, we would arrive at the fantastic plasma bilirubin concentration of 43 mg. per 100 ml. However, the potential bilirubin can easily be reduced to less than 200 mg. if a single, two-blood volume exchange is performed at birth. Such a transfusion will remove 85 to 90 per cent of the baby's red cells. Since bilirubin is also affected by a variable rate of excretion and production, such exact calculations are of little practical value in predicting the eventual height of the plasma bilirubin.

The pathogenesis of kernicterus has not been completely explained.

There is ample proof that kernicterus occurs in association with high plasma bilirubin levels and can be prevented by doing exchange transfusions which keep the bilirubin level below 20 mg. per 100 ml. Of course this does not prove a cause and effect relationship between the two. There has been considerable speculation about the relative danger of direct- versus indirect-acting bilirubin. In our opinion it is only the indirect-acting bilirubin that we need be concerned with.

Day[12] reported that bilirubin added to chopped rat brain in vitro produced a reduction in oxygen utilization. Waters[27] has produced kernicterus in newborn rats by the intraperitoneal injection of bilirubin. This did not occur, however, in adult rats. Such experimental evidence added to the clinical observations linking kernicterus to hyperbilirubinemia supports the theory that kernicterus is caused by bilirubin. Judging from the experimental work of Waters and the clinical observation that kernicterus occurs only in the first days of life, we assume that there is some difference in the newborn which allows kernicterus to occur.

From the practical standpoint it is almost certain that no baby will get kernicterus if the serum bilirubin level is kept below 20 mg. per 100

TABLE 7. *Average Serum Bilirubin in Various Groups of Infants*

	CORD BLOOD	1 DAY	2 DAYS	3 DAYS	4 DAYS
12 normal "compatible" infants	2.2 (1.5–3.2)	5.7 (3.4–7.4)	7.1 (3.1–12.4)	6.6 (0.6–13.2)	4.9 (0.0–12.3)
12 normal "incompatible" infants	2.0 (1.1–5.0)	5.7 (2.9–10.4)	6.5 (0.0–11.5)	5.8 (0.0–11.6)	4.5 (1.2–11.9)
15 premature infants under 2250 gm.	1.3 (0.0–2.9)	5.4 (0.0–10.3)	8.1 (0.0–15.3)	10.7 (0.0–21.6)	11.2 (0.6–27)

Figures in parentheses represent range. "Compatible" infants are those with no A, B or Rh incompatibility between infant and mother.

ml., a figure first quoted by Vaughn, Diamond and Allen[3, 26] and now confirmed by many other workers. It is reasonable to say that, with expert medical care, no baby should die (nor have irreparable brain damage) from kernicterus due to erythroblastosis. In theory, then, the indication for doing an exchange transfusion to prevent kernicterus is simple. It can be stated thus: An exchange transfusion should be done whenever the serum bilirubin level rises above 20 mg. per 100 ml. or whenever it is obvious that it will rise above 20 mg. In practice, of course, it is never quite this easy.

The following facts will be helpful when one is trying to decide whether an exchange transfusion needs to be done to prevent kernicterus.

1. Data on Bilirubin Levels. The serum bilirubin values during the first four days as determined by Hsia[17] are shown in Table 7.

2. The bilirubin level begins to fall spontaneously between 60 and 72 hours of age in the full term baby and on the fourth or fifth day in the premature baby.

3. After an exchange transfusion the bilirubin level will usually reach, or almost reach, the pretransfusion level eight to 12 hours after completion of the transfusion. This rise is due to the redistribution of bilirubin from the extravascular to the intravascular space.

It is possible to prevent hyperbilirubinema in two ways. We can remove the baby's red cells, thus preventing the production of bilirubin, or we can remove bilirubin after it has been formed. In theory, the first way should be the more effective, since about 85 per cent of the baby's potential bilirubin can be removed as red cells. We have evidence that there is a large store of bilirubin in the extravascular space. Brown, Zuelzer and Robinson[9] have shown in severely jaundiced babies that the total amount of bilirubin removed during the exchange transfusion may actually be more than was contained in the circulating blood volume at the beginning of the procedure. These experiments confirm the clinical impression that there is a large store of extravascular bilirubin which is in rapid equilibrium with that present in the plasma. In fact, the same authors report that a five- to 10-minute waiting period during the course of the exchange transfusion will result in a significant rise in the plasma bilirubin level. These observations make it evident that if one is doing an exchange for the purpose of removing bilirubin, the best results will be obtained by a slow exchange. A point of diminishing return will not be reached with as small a volume as with the exchange transfusion done to remove red cells. Thus a three-volume exchange will be considerably more effective than a two-volume exchange. Still more effective would be three one-volume exchange transfusions done at six to eight hour intervals.

If we assume that removing red cells before they have been hemolyzed is better than removing bilirubin, it becomes obvious that the best time to do the exchange transfusion for the prevention of hyperbilirubinemia is immediately after birth. One could do an exchange transfusion on all babies who have a positive Coombs test, but one would then be doing a large number of unnecessary transfusions. This, we feel, is completely unjustified with our *present* knowledge of the disease. How, then, do we decide which of these babies should get an immediate transfusion? We know of no points in the history, clinical symptoms or physical findings which are helpful, except possibly the development of clinical jaundice in the first two to four hours after birth. Such things as previous maternal and sibling history and maternal antibody titer are helpful as warning signs (i.e., they mean one should be prepared), but they do not answer this question in individual cases.

According to Boggs and Abelson,[6] a determination of the heme pigments is of value in predicting the need for an exchange transfusion. At present this determination is not available in most hospitals and is

difficult to do accurately. Certain observations indicate that the hemoglobin level can be used in this way. The statistics from the English combined study reported by Mollison[21, 22] showed that when using a single exchange transfusion in a full term baby there was no difference between those treated with multiple small transfusions and those treated with one exchange transfusion if the cord hemoglobin was above 11 gm. We know there are a few babies with hemoglobin levels between 15 and 20 gm. who do get kernicterus. However, the number of these is small, and kernicterus can be prevented in these by careful observation during the first four or five days of life.

From the data of Mollison and our own observations we think a cord hemoglobin below 11 gm. is an adequate reason for doing an immediate exchange if no other criteria are available.

Observations at The Children's Hospital indicate that the early degree of bilirubinemia is of value in predicting the future level of the serum bilirubin. These data showed that 80 per cent of those babies who had a cord bilirubin above 4 mg. per 100 ml. eventually needed an exchange transfusion, whereas only 20 per cent of those babies with a cord bilirubin below 4 mg. needed an exchange transfusion. In our experience we rarely need to exchange a baby whose bilirubin level is below *4 mg.* at birth, *7 mg.* at six hours or *10 mg.* at 12 hours. In babies whom we have "watched" it is seldom necessary to do an exchange transfusion when the rate of rise of indirect bilirubin is less than 0.23 mg. per hour on samples taken eight to 12 hours apart.*

In recent years we have had a gratifying decrease in the proportion of late cases referred to us. However, we still see some babies who come to us after severe jaundice has developed. The delay is sometimes due to a falsely negative Coombs test. This error has resulted in several fatalities and cases of kernicterus and is the bugaboo of the doctor who must rely on a laboratory where a Coombs test is done occasionally or by inexperienced personnel. We advise such a laboratory to include a positive Coombs control with their routine Coombs test.†

* If the physician detects a discrepancy between his estimate of jaundice and the laboratory results, he should insist on the inclusion of a known standard of bilirubin in the laboratory procedure. Our attention was drawn to the predicament of one of our former residents practicing in a small community hospital who had to make decisions on the basis of laboratory reports of the serum bilirubin which turned out to be 300 per cent higher than they should have been! A recent article by Hsia et al.[18] describes simple, rapid techniques for the determination of bilirubin in capillary blood.

† This is easily done by preparing one additional tube as follows: Place in the tube the amount of baby's blood ordinarily used in the direct Coombs test, add 2 drops of saline, and one drop of slide test (albumin active) anti-D typing serum. Let the tube stand for 5 or 10 minutes, then proceed to wash the cells in both tubes 3 times, and add antiglobulin serum to both tubes in the usual manner. If both tubes show agglutination, the baby has erythroblastosis. If only the control tube shows agglutination, the Coombs test is reported as negative. If neither tube shows agglutination, either the Coombs test is incorrect or one is dealing with an Rh-negative (cde) baby. The

In summary, we can say that the "rule of thumb" indications for doing an exchange transfusion are: First, evidence of erythroblastosis (i.e., positive Coombs test) plus one of the following:

1. AT BIRTH
   a. Clinical evidence of severe disease (large liver and spleen, pallor, poor respirations, edema, and so on) with or without frank signs of heart failure
   b. Cord hemoglobin below 11 gm. (14 in prematures) or a cord bilirubin level above 4 mg. per 100 ml.
2. A REPEAT EXCHANGE OR LATE FIRST TRANSFUSION
   a. Continued signs of heart failure or severe illness
   b. Bilirubin level higher than
      6 mg. per 100 ml. in first six hours,
      10 mg. per 100 ml. at 12 hours, and
      20 mg. per 100 ml. at any time

We do not regard such things as high maternal antibody titer, prematurity, and a family history of a previous severely affected infant as indications for an exchange transfusion. To do so is to reason with a "guilt by association" logic. We do consider them warning signs. When they are present, one should be prepared for rapid action.

The baby with erythroblastosis is in danger from hyperbilirubinemia until the serum bilirubin level begins to fall. This usually occurs in the full term baby at the beginning of the fourth day and not in the premature baby till the fifth or sixth day. Therefore it is of utmost importance to watch these babies carefully in the first three or four days even though they do not appear ill and have already had one or two exchange transfusions. Depending on the height of the original bilirubin determination, the clinical appearance, the degree of jaundice, and the like, it may be wise to repeat the bilirubin determinations as often as every eight to 12 hours. Certainly careful clinical observation should be made at least every eight hours. A "late" exchange should be done if the serum bilirubin threatens to rise above 20 mg. per 100 ml. The only exception to this might be when the bilirubin level is rising slowly and has reached this level at the time a spontaneous fall can be expected, i.e., at the end of the third day in a full term baby.

The baby's behavior pattern is of considerable help in spotting early kernicterus; this pattern is present at a time when an exchange transfusion may reverse the process. This behavior, we realize, is not specific

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infant's cells should give a positive control test with anti-D antiserum in all cases of D sensitization and all except rare instances of sensitization to the other Rh and Hr factors. For all practical purposes, the only cells of a jaundiced newborn baby which would not give a positive control test would be those of an Rh-negative (cde) baby with A or B sensitization or severe physiological jaundice. For the management of such infants, see the following sections. The mother's cells should always give a negative result of the direct Coombs test and, if this is included in the initial battery of tests, should provide a valid "negative Coombs control" and an insurance against cross-labelling of the baby's and the mother's blood specimens.

for kernicterus, since it may be present in other babies. However, when it occurs in a baby who has erythroblastosis, an immediate exchange transfusion should be done. The baby tends to be lethargic. When left undisturbed, or if casual things are done such as lifting the blanket or touching him lightly, he lies quietly in his crib and does not make the stretching, catlike, random movements characteristic of little babies. However, when disturbed by a light tapping of his foot or a soft quick movement of the hand over his abdomen, he has an abnormal and exaggerated Moro response. This he will do to a stimulus that does not cause a Moro response in the average baby. This Moro is abnormal and incomplete. The arms go out to the side, the fingers extend, and the Moro usually culminates in tremors of the arms, hands and fingers. The arms and hands do not come around in a grasping movement to complete the Moro.

We have found the following signs of no value: vigorous kicking and crying, character of cry, color, heart tones, respirations and appetite. For example, we have seen a baby nurse well up to within two hours of death due to kernicterus. Convulsions, opisthotonos, respiratory disturbance, hemorrhage, are all signs of late kernicterus. When they are present, it is too late.

ERYTHROBLASTOSIS DUE TO THE SO-CALLED RARE BLOOD FACTORS

The physician must be prepared for the occasional, early jaundiced baby of an Rh-positive mother. The nursery supervisor will bring such babies to his attention if he has taught her that "jaundice appearing before 24 hours of age is always abnormal and almost always means that the infant has erythroblastosis." All such babies should have an immediate Coombs test, and the mother's and baby's bloods should be typed completely. Finding an Rh-positive infant who has a positive Coombs test by no means indicates that his mother is Rh-negative and that his erythroblastosis is due to the usual factor D. Actually, almost every baby affected by an Rh or Hr antibody is Rh-positive (D). The discovery that the mother of such a baby is Rh-positive (D) is usually the first indication that the erythroblastosis is due to a factor other than the usual D. In such situations it should be remembered that erythroblastosis occurs only in a heterozygous baby of a homozygous mother. The mother must be homozygous, lacking the factor involved, because she cannot produce antibodies against a blood group factor which her cells contain. The baby, on the other hand, must be heterozygous or he will not stimulate the mother to produce antibodies against his own cells.

The commonest situation of this type is that of the mother homozygous for factors C, D, e, who bears a baby heterozygous for factor

C (CDe/cde). In this case the mother would have formed anti-c. If the baby were heterozygous for factor E, antibody might be formed against E as well as or instead of c. Inspection of Table 8 will illustrate the most common possibilities encountered.

These are rather common, theoretical mother-baby incompatibilities. The explanation for the rarity of such combinations lies in the poor antigenicity of the factors other than D. The great increase in transfusions given to girls and women before the childbearing age will result in an increase in this type of sensitization.

In such instances the baby's cells are Rh-positive and Coombs-positive, and the clinical course of the baby's disease is entirely compatible with what might be expected with the usual D erythroblastosis. Indications for exchange transfusions are identical with those of D erythro-

TABLE 8. *The Most Frequent Blood Type Incompatibilities Allowing the Development of Uncommon Antibodies Responsible for Erythroblastosis.**

MOTHER'S TYPE (HOMOZYGOUS)	INFANT'S TYPE (HETEROZYGOUS)	POSSIBLE MATERNAL ANTIBODIES
CDe/CDe	CDe/*cde*	anti-c
CDe/CDe	CDe/*cDE*	anti-c, anti-E
cDE/cDE	cDE/*cde*	anti-e
cDE/cDE	cDE/*CDe*	anti-e, anti-C
Kell neg./Kell neg.	Kell neg./*Kell positive*	anti-Kell

* For additional possibilities, see Allen and Diamond.[2]

blastosis, but the selection of donor blood must be such that the same type of cell is used as the mother's.

If the laboratory has commercially available subtyping serums anti-C, anti-E and anti-c, most patients with the above types of sensitization can be investigated quickly by subjecting mother's and baby's cells to agglutination by these serums to determine whether the mother is homozygous and the baby heterozygous for the C and E factors. Donor bloods may be selected from the blood bank to give the same reaction as the mother's cells. Of course any donor may be used to transfuse the baby whose cells show no agglutination by a correctly performed indirect Coombs test with the mother's serum if the mother's antibodies are strong. Use of the mother's serum for this purpose may lead to error if the titer of her antibodies is not high. Consequently it is usually better to proceed with selection of the donor with commercial serums and then to confirm the selection with the indirect Coombs test, using donor's cells and mother's serum.

Any laboratory servicing a moderate to large obstetrical service should be able to obtain quickly group O cells known to be CDe (Rh_1), cDE (Rh_2) and cde (Rh-negative). If Rh-negative cells can be located which are Kell-positive, they are even more useful. These three cells

can be tested with the mother's serum by the indirect Coombs method. Table 9 indicates the specificity of the mother's antibody when these cells are used.

Even more complicated panels of cells may be designed, but the above cells are usually available among the personnel of even a small

TABLE 9. *Reactions with Red Cells of Known Subtypes Which Indicate the Specificity of Maternal Antibody*

TEST CELL			
CDe k	cDE k	cde K	SPECIFICITY OF MOTHER'S ANTIBODY
+	+	−	D
+	−	−	C
−	+	−	E
−	+	+	c
+	−	+	e
−	−	+	K
+	+	+	Cold aggl. or possibly Duffy, Kidd, etc.

hospital, and previous subtyping of such people offers a practical solution to the laboratory determined to be prepared to do a good job in this type of work.

A-B ERYTHROBLASTOSIS

The frequency with which A-B erythroblastosis is diagnosed bears a direct relationship to the intensity of the search for it. This is illustrated in Table 10. The striking increase in ABO erythroblastosis in 1955 is explained by the concerted effort which was made to explain all cases of early jaundice in that year.

TABLE 10. *Frequency of Diagnosis of Rh and AB Erythroblastosis Made at the Ohio State University Health Center*[10]

	ABO	Rh
June-December, 1953	3	9
January-December, 1954	4	20
January-December, 1955	18	26

Little change of thought concerning the nature of A-B erythroblastosis has occurred during the past few years. The antibody responsible for the disease in the infant is indeed different from the "natural" anti-A or anti-B antibody which produces agglutination of A or B red cells in saline suspension. The disease-producing antibody resists neutralization by commercial A and B substances, but agglutinates A_1 or B cells in colloid media. This so-called immune antibody may be eluted

from the affected infant's red cells, whereupon it can often be shown to cross react with A_1 or B cells. The most likely explanation of this phenomenon is that such antibody reacts with a portion of human A_1 substance or B substance which is not present in commercial material and has little to do with the actual immunological difference between A and B polysaccharide, but which is common to both.

Unless a physician is alerted to the possibility of A-B erythroblastosis by a history of previous affected siblings, the first awareness of the disease will come from the observation of jaundice developing during the first day or two of life. It frequently occurs in first babies. Serologic tests show the baby's cells to be Coombs-negative or to give at most a 1 or 2 plus reaction, and the mother's serum to be lacking in antibodies to the Rh, Hr or the "rare" blood group factors. In our hands the most consistent serologic confirmation of the disease is the demonstration in the baby's serum of antibodies which, after neutralization with commercial A and B substance, will give a positive indirect Coombs test with *adult* A_1 or B cells, but not A_2 or O cells.

The physician will soon learn that laboratory confirmation of this type of hemolytic disease does not bear the precision, speed or validity of tests for Rh erythroblastosis. He may find himself faced with a decision of whether or not to exchange a baby with early and rapidly rising jaundice on whom he has no more information than that the mother is type O and the baby type A or B with a negative Coombs test. As mentioned later, it is of academic interest only whether the jaundice is due to an extreme example of physiological jaundice or of A-B erythroblastosis. A serum bilirubin level allowed to rise higher than 20 mg. per 100 ml. and to remain at that level in the first five days has been demonstrated to carry a significant risk of the development of kernicterus. The principles of management of such a baby are identical with those outlined for Rh erythroblastosis except for the selection of donor blood, which should be O, and of the same Rh type as the baby. Such a baby should be exchanged without apology.

Most workers agree that 10 cc. of commercial A and B substances should be added to the pint of donor blood. In our opinion it is more important to select donor blood which has less than a 1:200 saline titer against A_1 or B cells, although we do use the A and B substances also.

JAUNDICE DUE TO CAUSES OTHER THAN ERYTHROBLASTOSIS

It has become increasingly apparent that brain damage may result from intense jaundice in the newborn period from a variety of causes. Zuelzer and Mudgett[30] pointed out that in prematures, especially colored prematures in whom jaundice was not readily apparent, ker-

nicterus was often found at autopsy. A study of these cases revealed no apparent causes for the jaundice other than the supposed immaturity of the premature liver. Other workers have since confirmed these findings. Bound and Telfer[7] recently pointed out a correlation in prematures between the administration of large doses of vitamin K (10 mg. daily for three days) and intense jaundice and kernicterus. Meyer and Angus[19] have confirmed these findings. Recently, Silverman[25] demonstrated high incidence of kernicterus and hyperbilirubinemia in prematures receiving Gantrisin plus penicillin for "routine" prophylaxis against infection.

Crigler and Najjar[11] have described one family and Rosenthal, Zimmerman and Hardy[23] another family wherein some newborns exhibited a defect in liver metabolism which permanently prevented efficient conversion of the indirect-reacting type of bilirubin to the direct-acting type. Many of these children had kernicterus.

Occasionally jaundice assumes kernicterus-producing proportions in newborns afflicted with congenital hemolytic anemia. Here replacement of the baby's red cells with those of a donor who does not possess the hereditary defect makes as much sense as in erythroblastosis. In addition, a splenectomy may be performed to minimize the tendency to hemolysis of the baby's freshly formed red cells.

Bacterial sepsis in the newborn, especially due to colon bacillus, is frequently associated with serum bilirubins above 20 mg. per 100 ml., and kernicterus has been described in such conditions.

It is our feeling that with the exception of the group described by Najjar, the jaundice should be controlled by exchange transfusion if detected before symptoms of kernicterus become manifest. Here the donor blood should be of the same group and Rh subtype as the infant to avoid the possible later development of blood group sensitization. Furthermore, since most of these babies will have been exchanged after the jaundice level has become threatening, less than optimal benefit may be derived from the procedure, and re-exchange may be found necessary. *Although control of serious jaundice by exchange transfusion in the situation just described seems reasonable, there have been too few data compiled to demonstrate conclusively the efficacy of this method of treatment.*

SUMMARY

This paper is not a comprehensive discussion of erythroblastosis. We have emphasized pathogenesis and the therapy, particularly therapy with exchange transfusion. The temporarily impaired bilirubin excretory function of the newborn's liver has been emphasized as a large factor in the production of intense jaundice.

The indications for an exchange transfusion reduced to the simplest terms are (1) to prevent death in a patient who has potential or actual cardiac failure, and (2) to prevent hyperbilirubinemia and the kernicterus it causes. The exchange transfusion is an excellent means of correcting or preventing the heart failure which is a common occurrence during the first day of life. Exchange transfusion is more effective in preventing hyperbilirubinemia than in treating it. Therefore an early exchange is wiser than a late one.

The principles of bilirubin control used successfully in erythroblastosis due to Rh incompatibility can profitably be extended to other forms of hemolytic disease and other diseases causing jaundice in the newborn.

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ABO HEMOLYTIC DISEASE AND HETEROSPECIFIC PREGNANCY

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The disease known as ABO hemolytic disease of the newborn has come to be universally accepted as an entity of a sort. It is a remarkable entity which has not been and perhaps cannot be adequately defined on either clinical or serologic grounds. The term itself embodies the current and perhaps too narrowly conceived theory of its pathogenesis and leads to a purely theoretical definition, that of a pathological condition caused by the action of maternal anti-A or anti-B antibodies upon fetal erythrocytes of the corresponding A-B blood group.

Out of the whole group of heterospecific pregnancies in which the maternal serum contains an antibody antagonistic to the fetal blood group, this definition somewhat arbitrarily lifts those cases in which the antibody appears to have a harmful effect on the offspring. It stresses the pathological aspects of processes which extend without a clear line of demarcation from the normal to the abnormal and which are probably more often than not without untoward effects. This rather one-sided definition omits all mention of characteristic clinical or laboratory features. It implies maternal iso-immunization in analogy to the mechanism in Rh hemolytic disease, but says nothing about the origin of the antibodies or the nature of the antigenic stimulus.

These omissions are deliberate and reflect the present state of our knowledge—or rather ignorance—concerning many aspects of ABO hemolytic disease. Despite careful studies documenting its existence, it has no pathognomonic serologic features. In its symptomatology it overlaps with so-called physiologic jaundice, with which it has often

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been confused, and with Rh hemolytic disease. Most cases probably go unrecognized even today and are so mild, so transient and so lacking in permanent sequels that one hesitates to label the condition a disease. Yet ABO hemolytic disease is no myth, but a reality which becomes frightening, since it may, if neglected, end much like Rh hemolytic disease with an encephalopathy, kernicterus, with subsequent death or development of grave neurological sequels. It is essential, therefore, to be on guard against such an event and to be familiar with the practical and theoretical aspects of the condition. Its very danger lies in its apparent mildness when regarded as a hemolytic process, in its deceptive blending into the bilirubin-tinted landscape of newborn physiology.

THEORETICAL CONSIDERATIONS

At the outset it may be useful to set forth the reasons why ABO hemolytic disease has been and continues to be so elusive, why it is a late-comer, accepted long after Rh hemolytic disease had become an established entity, although the discovery of the ABO blood groups preceded that of the Rh factor by nearly 40 years, and despite the fact that the role of the A-B blood groups in neonatal jaundice was suspected long ago.

ABO hemolytic disease is a special event connected with heterospecific pregnancy and must be viewed against the background of the full range of phenomena associated with such pregnancies. The term "heterospecific pregnancy" designates situations in which the maternal serum contains an iso-antibody antagonistic to the fetal blood group. The following types of pregnancy fall into this category:

Mother A—Infant B
Mother B—Infant A
Mother O—Infant A or B

Actually, the occurrence of ABO hemolytic disease is virtually limited to the last of these combinations, to the A or B offspring of group O mothers. This fact, which Rosenfield[15] was the first to point out, reduces the incidence and was therefore a source of confusion for earlier workers who were looking for a pattern common to all heterospecific pregnancies. Failure to recognize the special role of the group O mother undoubtedly delayed recognition of the entity. We shall return to this important point later on.

A more difficult problem is posed by the "natural" occurrence of anti-A and anti-B antibodies in normal persons. By definition such antibodies are present in every heterospecific pregnancy. Their appearance in the maternal serum dates back to the mother's early childhood and is clearly unrelated to the event of pregnancy, transfusion or exposure to human blood group substances of any kind. Since most

heterospecific pregnancies do not seem to lead to hemolytic disease, the presence of anti-A or anti-B in the maternal serum is not of pathological significance and can therefore not be of diagnostic significance when ABO hemolytic disease is suspected.

Compare this situation with the presence of Rh antibodies in the maternal serum. A difference in the Rh types of mother and child is not automatically associated with the presence of a maternal Rh antibody. When such antibody is found, it always has pathological and usually diagnostic significance because it clearly denotes specific immunization with a human antigen which can occur only as the result of exposure during pregnancy or by blood transfusion. In a fetus of susceptible Rh type the maternal Rh antibodies almost invariably produce some degree of disease in the offspring.

The existence of the so-called natural anti-A and anti-B antibodies in normal serums thus poses both a practical and a theoretical problem. The mere demonstration of anti-A or anti-B in the maternal serum has no additional diagnostic value over that of establishing that the blood groups of mother and child are incompatible. And the failure of these antibodies to cause hemolytic disease in most instances of heterospecific pregnancy needs to be explained just as much as their assumed ability to do so in a minority of cases. Finally, the origin of the anti-A and anti-B antibodies, the antigenic stimulus which calls them forth, is usually not known and may not be related to the events connected with pregnancy.

These difficulties have led to a search for special mechanisms along two lines: On the one hand, students of the problem have looked for protective devices in the fetal organism or placenta which might render the maternal antibodies normally present in heterospecific pregnancy harmless for the average infant. On the other hand, investigators have attempted to demonstrate special kinds of anti-A or anti-B antibodies, often referred to as "immune" as opposed to "natural" iso-agglutinins in order to explain the special case of ABO hemolytic disease. It is likely that both kinds of arguments have to be invoked to explain the observed facts.

Fetal Protective Mechanisms

Along the line of the first argument an early theory suggested the secretor status of the offspring as a protective mechanism. So-called secretors have appreciable amounts of A or B blood group substances in soluble form in their plasma, saliva, gastric juice and tissue fluids. The secretor property appears to be fully developed at birth. The presence of free soluble blood group substances was thought to neutralize the maternal antibody before it could reach the erythrocytes. Accord-

ingly, nonsecretors, having no such protection, should be liable to ABO hemolytic disease by analogy with the situation in Rh hemolytic disease in which the erythrocytes are always vulnerable because, with respect to the Rh factor, every person may be regarded as a nonsecretor.

In its original form the secretor-nonsecretor hypothesis could not be maintained. It was shown that secretors as well as nonsecretors could be affected by hemolytic disease, and that the proportions of one group to the other were not far from those expected in a random sample of the population. Still, the possibility has not been ruled out that the secretor status provides some protection, a relative protection which can be overpowered by potent maternal antibodies, but may be sufficient to avert hemolytic disease in the average case of heterospecific pregnancy.

However, nonsecretors as well as secretors have large amounts of specific blood group substances in the tissue cells themselves, and it is probable that these cellular substances play a more important part than the secreted substances in neutralizing or fixing antibody. The tissue elements contributed to the placenta by the fetal organism have specific blood group substances like the rest of the fetal tissues and are likely to be particularly important, because it is here that the maternal antibodies first come in contact with fetal blood group substances.

The following observations suggest that the fixation or neutralization of antibodies by tissue A or B substances may be an important and ordinarily adequate device for the protection of the fetal erythrocytes in heterospecific pregnancy: If one studies for comparison homospecific pregnancy of the type mother O-infant O, in which there is no fetal substrate with which the maternal anti-A and anti-B antibodies can react, one finds that both these antibodies can usually be demonstrated in the cord blood. The cord blood titers are lower, but roughly proportionate to the maternal titers. Evidently the placenta is permeable to ordinary anti-A and anti-B antibodies, since it cannot be assumed that the fetal organism itself produces such antibodies. In heterospecific pregnancy of the type mother O-infant A or B the antibody specific for the infant's blood group is normally lacking in the cord serum, but the heterologous antibody is present. For example, the cord blood of a group A infant will show no anti-A, but will contain anti-B. In a group B infant anti-A is present as expected, but anti-B is lacking.[16, 27]

There is no a priori reason to believe that the structure and permeability of the placenta in heterospecific pregnancy are at all different from what they are in homospecific pregnancy. It follows that both antibodies of a group O mother pass through the placental barrier in

either type of pregnancy, but that in heterospecific pregnancy the antibody antagonistic to the fetal blood group is bound in some manner on the fetal side by specific fetal blood group substances while the opposite antibody remains free. Since the fixation of antibody occurs in nonsecretors as well as in secretor infants, it must take place within the cells, and since hemolysis is not normally present, tissue cells rather than erythrocytes must be involved. There is no evidence that this hypothetical antigen-antibody reaction involving tissue substances ordinarily has a harmful effect on the fetal organism, although some European workers have considered a toxic effect of this kind in the pathogenesis of ABO hemolytic disease.[8]

Another possible protective mechanism may reside in the relatively low reactivity of fetal erythrocytes for antibodies of the ABO system.[18] Evidently this factor is a variable which has not been accurately assessed. That it is likely to play a role in determining the effects of maternal antibodies is suggested by several observations: The hemolytic activity, the agglutinin titer and the avidity of anti-A or anti-B antibodies are generally weaker when tested against newborn than adult erythrocytes. The development of the A_1 antigen is not always completed at birth,[24] so that the erythrocytes of about half the infants later found to belong to subgroup A_1 initially give the reactions of subgroup A_2. Evidence has been accumulating that in ABO hemolytic disease the maternal antibody has anti-A_1 specificity and that the affected children of group A belong to subgroup A_1. Infants of subgroup A_2 seem to escape altogether. For B the situation is not so clear cut, since no sharp distinction between subgroups of this blood group has been established.

Apart from the mechanism specifically related to the reactions between antibodies and blood group substances, it is obvious that nonspecific factors influence the development and even the recognition of hemolytic disease. It is apparent to the clinician that jaundice, which is the predominant symptom of ABO hemolytic disease, and the hyperbilirubinemia which constitutes its greatest danger, are not always proportional to the load imposed by the intensity of the hemolytic process. Some infants with minimal anemia are severely jaundiced, others with severe anemia seem to handle the excretion of bilirubin satisfactorily. The same is true for Rh hemolytic disease and even for so-called physiologic jaundice in the absence of abnormal hemolysis. Variables unrelated to the hemolytic process evidently must play a part in determining the manner in which the bilirubin load is handled. It is becoming increasingly apparent that the chief variable resides in the ability of the liver to excrete bilirubin, and this function seems to be an enzymatic one, probably involving the coupling of "free," indirect bilirubin with glycuronic acid. In infants in whom this process is well

handled the presence of hemolysis may go unnoticed, while others may not tolerate even a normal load.

It is important to realize that the consequences of the hemolytic process for the newborn infant depend at least in part on factors which do not seem directly related, and may not be related at all to the underlying cause of hemolysis, but to the speed and efficacy of adaptive functions in the metabolism of the newborn. It is this variability of unrelated factors which makes it difficult to define ABO hemolytic disease on clinical grounds, to decide where a more or less well tolerated hazard inherent in the physiological mother-child relationship ceases to be physiologic, at what point it transcends the boundaries of normal adjustments and becomes a disease.

Anti-A and Anti-B Antibodies

Regardless of the importance of the various specific and nonspecific mechanisms which may contribute to the protection of the fetus, the maternal antibodies themselves may be expected to play a large role in determining whether heterospecific pregnancy has a normal outcome or leads to hemolytic disease. From the beginning special attributes of the maternal anti-A and anti-B iso-antibodies have been looked for in cases of ABO hemolytic disease. The expectation of a simple quantitative relation between the height of the antibody titer and the development of hemolytic disease did not materialize. Though in some instances exceptionally high maternal saline agglutinin titers are found and in the group as a whole the titers are higher than in normal control groups, many individual cases show titers well within the normal range. It was necessary, therefore, to look for qualitative differences between ordinary, so-called natural iso-agglutinins and so-called immune anti-A and anti-B antibodies.[1, 3, 23]

A precise definition of immune as opposed to natural iso-antibodies of the ABO system has not been achieved; indeed, the validity of such a distinction may be challenged on theoretical grounds. A considerable body of opinion exists today which considers that the maternal iso-agglutinins, like all antibodies, are the result of contact with antigen, are an expression of immune processes.[10] The wide distribution of the A and B antigens in nature outside the human race, in microorganisms, plants and animals, provides the opportunity for exposure. Natural iso-agglutinins may be interpreted as the response to these cross-reacting heterogenetic antigens. Various immunizing procedures with substances of bacterial or animal origin can produce "immune" responses of the anti-A and anti-B antibodies, similar to those following injection of human blood or blood group substances.

Whether or not one chooses to make a theoretical distinction be-

tween natural and immune iso-antibodies, empirically it must be recognized that the anti-A or anti-B antibodies of certain serums, coming mainly from group O persons, may have special properties not present in the majority of serums. These properties are commonly referred to as immune. Briefly, such antibodies, unlike ordinary α and β agglutinins, may show one or more of the following features:

1. A thermal optimum of 37° C. Ordinary agglutinins react best at lower temperatures.
2. Greater resistance to heating at 70° C.
3. "Conglutination" in colloid media such as serum or gum acacia with higher titers than obtained by agglutinin action in saline medium
4. A positive Coombs (antiglobulin) test at dilutions well beyond the end point of titration in saline
5. Resistance to neutralization by soluble specific blood group substances A or B which will neutralize the saline agglutinins. The test is carried out by titration in AB serum or by using the indirect Coombs test after the antibodies have been partially neutralized with A and B substance.
6. Hemolysis and complement fixation in vitro

These properties came to light for the most part in investigations of transfusion reactions following the use of group O blood for recipients of group A or B. Such group O donors are often referred to as dangerous universal donors.[4] Those originally studied were males and nulliparous women who had not undergone recent immunizing procedures of any kind. The behavior of the anti-A or anti-B antibodies in such persons may remain unchanged for years. The nature of the antigenic stimulus which calls forth and maintains such a response is not known.

Serums of group O mothers whose infants have ABO hemolytic disease frequently exhibit one or more of these "immune" anti-A or anti-B reactions, behave, in short, like the serums of dangerous universal donors. Since this pattern occurs in certain O serums in the general population, male and female, it is clear that it is not necessarily caused by heterospecific pregnancy or blood transfusion or injection of human blood group substances. Whether pregnancy itself can be a significant antigenic stimulus transforming "natural" antibodies into "immune" antibodies or increasing already existing antibodies of the "immune" type and thereby lead to ABO hemolytic disease in a subsequent child is not a securely established point. Few systematic studies of this question have been made. Postpartum rises in titer, development of hemolysins and other "immune" properties have been observed.[27] Hemolysins are more frequently observed in multiparous than nulliparous women, according to some authors.[9] But these responses may be transient and may not significantly add to the pre-existing normal or abnormal state of immunity with respect to A and B antigens. The patterns of anti-A and anti-B of pregnant group O women who had previously borne normal group A or B children were not found signifi-

cantly different from those of group O women who were either nulliparous or had borne only group O infants.

Whether or not pregnancy is ever a cause of anti-A or anti-B iso-immunization, it is clear that it cannot be the only one. Conversely, however, one might regard mothers whose infants have ABO hemolytic disease as dangerous universal donors whose antibodies, whatever the stimulus, will exert their effect on the substrate of fetal erythrocytes, provided the fetus happens to belong to a susceptible blood group.

This concept would explain why ABO hemolytic disease often affects the first-born child or the first A or B child born to a group O mother. The striking contrast of this pattern with the well known pattern of Rh hemolytic disease can be understood only if one recognizes the role of heterogenetic antigens serving as a stimulus for the immunization of the mother prior to pregnancy.

The "immune" character of the iso-antibodies of dangerous universal donors—and the mothers of infants with ABO hemolytic disease—would serve to explain some of the observed facts. The "immune" antibodies are regarded by some authors as "univalent" or incomplete antibodies capable of passing the placental barrier more readily than the "bivalent" ordinary saline agglutinins.[22] The failure of the "immune" antibodies to be neutralized in vitro by soluble A or B substances[23] might indicate a similar failure in vivo and would logically explain their ability to attack the fetal erythrocytes.

Unfortunately, not only is the demonstration of "immune" anti-A and anti-B iso-antibodies a relatively cumbersome laboratory procedure which makes diagnostic application rather difficult, but also the correlation between their presence in the maternal serum and hemolytic disease is far from satisfactory. On the one hand, they may be lacking in cases of ABO hemolytic disease which otherwise seem to meet the diagnostic criteria. On the other hand, the maternal serum may show an "immune" pattern, yet the infant may appear entirely normal. The problem is multiplied by the practical difficulties in characterizing an "immune" anti-A or anti-B. As might be expected from the theoretical considerations given above, there are no all-or-none differences between natural and "immune" antibodies. Many normal serums show some degree of hemolytic activity, a one plus or 2 plus reaction. In many serums one may find antibodies not completely neutralized by A or B substances. Is a residual titer after neutralization to be regarded as significant when it is low—for example, in the range of 1:8 to 1:16? Does it become significant when it exceeds 1:32? Similarly many serums show somewhat higher titers in colloid media than in saline. How many units of titration make the difference significant?

But the greatest difficulty lies in the fact that the various "immune" properties do not combine in any consistent pattern. Some serums

show hemolysins but no antibodies resistant to neutralization, others the reverse; still others may contain conglutinins, but fail to give most of the other reactions. It is not only a theoretical problem to say which property is truly indicative of an "immune" antibody, whether the demonstration of any one suffices to characterize the serum as "immune" or whether all must be present; it is also difficult from a practical standpoint to select a representative test or group of tests for diagnostic purposes.

Even the specificity of the anti-A or anti-B iso-antibodies in the maternal serum as in group O serums in general is in doubt. Frequently in ABO hemolytic disease both antibodies show an elevated titer or have "immune" properties regardless of the blood group of the afflicted infant. In some instances the antibody specific for the baby's blood may even appear less potent than the heterologous antibody. Cross reactions have been observed in serums of group O mothers with affected offspring, as in some random group O serums, as shown by lowering of the anti-A titer after absorption with group B erythrocytes, and vice versa. When erythrocytes of one blood group are sensitized in vitro by inoculation in such serums, the antibody eluted from these cells may agglutinate erythrocytes of the opposite blood group.[17] Anti-A and anti-B in the cord serum of group O infants can often be cross-absorbed.

It is likely that these phenomena, peculiar as they are to group O serums, have some connection with the almost exclusive occurrence of ABO hemolytic disease in children of group O mothers, but the exact meaning of this relationship is still obscure. One theory goes so far as to assume that the offending antibody in both group A and group B infants is a cross-reacting antibody, labelled anti-C, which is directed against an antigenic factor "C" common to the agglutinogens A and B, but lacking in group O erythrocytes and therefore produced only by group O individuals.[19, 21]

A less radical hypothesis assumes that the incompatible fetus of a group O mother may be subject to the effects of more than one antibody, cross-reacting as well as that specific for its blood group. It must be realized that cross-reacting antibodies are not invariably present and that in rare instances hemolytic disease does develop in A infants of group B mothers and B infants of A mothers when only specific antibody can be involved.

What can be the meaning of this variability in maternal antibodies? There is reason to believe that the nature of the antigenic stimulus as well as differences in individual response plays a role in determining the pattern of anti-A and anti-B iso-antibodies in a given person. Human antigens may produce somewhat different responses from those caused by heterogenetic antigens. The ubiquity of A and B substances in nature may lead to successive exposures with slightly different or identical

antigens, producing an infinite number of variant responses, with antibodies of different partial specificity and different properties in vitro. The mode of contact may play a part. And inherited determiners for the ability to produce antibodies may be involved. All in all, the complexity of the antigen-antibody relationships in the ABO system is such that a uniform pattern cannot be expected.

If this viewpoint is correct, it is indeed unlikely that tests involving the maternal serum can ever be as informative for ABO hemolytic disease as those developed for Rh hemolytic disease. One may doubt that a clear-cut diagnostic picture of the maternal anti-A and anti-B antibodies exists. Rather, the spectrum of maternal antibody patterns, combined with the variability in fetal protective mechanisms, explains both the many combinations and permutations of the clinical manifestations which can be associated with heterospecific pregnancy and the lack of a diagnostic pattern. Thus the clinical definition and the laboratory diagnosis of ABO hemolytic disease, although it may be supported by study of the maternal antibodies, must both be based on other criteria.

The ideal diagnostic criterion would be the demonstration of antibody coating the fetal erythrocytes. In Rh hemolytic disease the direct antiglobulin test serves this purpose and has become the keystone for its diagnosis. As for ABO hemolytic disease, this test has given variable and often negative results. As performed in the standard manner with commercial antiglobulin serums available in this country, it is positive at most in 50 per cent of the cases and is usually weak, at most one plus to 2 plus, more often only microscopically positive. This unreliability or failure of the direct Coombs test appears to have its basis in the weak reactivity of the neonatal A and B antigens.

That this explanation is correct is substantiated by the fact that frequently antibody can be eluted from fetal erythrocytes which themselves give a negative direct Coombs test. Such antibody can be demonstrated by the indirect Coombs test with adult erythrocytes. Moreover, the serum of the afflicted infants usually contains free antibody which reacts with adult erythrocytes, even though the direct Coombs test of the infant's own erythrocytes may be negative or at most weakly positive.

A more sensitive modification of the Coombs test than the standard procedure has been perfected by Rosenfield, who showed that with this modification a positive reaction can be consistently obtained in ABO hemolytic disease.[16] The difficulty in applying this method is in part technical in that the reactions are frequently so weak as to require precise microscopic readings. A greater problem is created, however, by the fact that the Rosenfield-Coombs test is positive in 11 per cent

of all incompatible infants of group O mothers, including many babies who appeared clinically normal.

The test, according to its author, detects cases of group incompatible infants whose mean reticulocyte count is slightly elevated and whose mean serum bilirubin level is slightly higher than that of a group of incompatible infants with a negative Coombs-Rosenfield test. The test therefore seems to correlate with minimal biologic effects of heterospecific pregnancy rather than with clinical hemolytic disease as does the standard Coombs test in Rh hemolytic disease.

In view of these difficulties, the infant's direct Coombs test is not unequivocally suitable for the diagnosis of ABO hemolytic disease even when its sensitivity is increased. Better correlation with clinical hemolytic disease was obtained in tests utilizing the serum of the infant and demonstrating the specific antibody against adult erythrocytes of the same blood group.

BIOLOGIC EFFECTS OF HETEROSPECIFIC PREGNANCIES

There is no question that heterospecific pregnancy is compatible with the normal implantation, development and delivery of the fetus. Some authors have noted a deficit of incompatible offspring in the pregnancy of group O mothers, notably in matings of the type father A with mother O,[20] and have interpreted their observations as evidence for the resorption of incompatible fetuses at an early stage of gestation. In other series, however, no such shortages of incompatible offspring have been found.[14] ABO incompatibility has also been linked to abortion and early miscarriage.[11] It has been stated that in cases in which the mother had two or more abortions the incidence of incompatible matings is higher than in the general population, but these findings have not been confirmed. At present the evidence that ABO incompatibility is a cause of early fetal death is inconclusive.

In the majority of cases heterospecific pregnancy is not associated with a demonstrable handicap for the newborn infant. Ordinarily neither a hemolytic process nor undue jaundice is observed. When they appear, they must be regarded as pathologic even though their clinical significance may be minimal.

Halbrecht was the first to note that infants who showed jaundice without evident anemia shortly after birth were usually incompatible with their mothers in the ABO system.[6] He coined the term "icterus praecox" for what is now recognized as a relatively mild form of ABO hemolytic disease. Over the years it was recognized that more typical hemolytic disease with frank anemia and other findings familiar from the picture of Rh hemolytic disease occurs on the basis of ABO in-

compatibility. Usually the hemolytic process is less spectacular than in Rh hemolytic disease. Stillbirths have been attributed to ABO incompatibility, but the number of authenticated cases is small.

As already mentioned, Rosenfield observed a slightly higher mean reticulocyte count and bilirubin level in incompatible infants whose erythrocytes gave a positive Coombs test by his method. A few of those patients had hemolytic disease, but the majority appeared clinically normal. The observations are evidence for the existence of a border zone in which the effects of heterospecific pregnancy on the fetus are minimal, yet no longer entirely "normal."

In keeping with this interpretation are the results of erythrocyte survival studies in infants who are produced by heterospecific pregnancy. Mollison and Cutbush[13] and Boorman, Dodd and Trinick[2] showed that in frank hemolytic disease due to anti-A the survival of transfused A erythrocytes is greatly shortened in comparison with that of simultaneously administered group O erythrocytes. These observations were needed at the time to prove unequivocally that the hemolytic process was specifically due to the maternal anti-A antibodies. Zuelzer and Kaplan[28] subsequently demonstrated preferential destruction of group A over group O erythrocytes in several infants who had appeared clinically and hematologically normal. Evidently many gradations in the effects of heterospecific pregnancy can occur, and there is no fixed boundary between the normal state and what may be called hemolytic disease. In each case the condition of the infant appears to be the resultant of the various maternal and fetal specific and nonspecific factors referred to above. For the same reason there can be no sharp distinction between the different abnormal manifestations attributable to heterospecific pregnancy, such as icterus praecox versus hemolytic disease, except for clinical convenience.

HEMOLYTIC DISEASE OF THE NEWBORN DUE TO ANTI-A AND ANTI-B

Because of the difficulties in recognition and definition discussed in the preceding sections, the incidence of ABO hemolytic disease is still not established. Until recently many cases were mistaken for physiological icterus, and even today the mild cases are often overlooked. Taking Rosenfield's series, which includes all infants with a positive modified direct Coombs test and therefore many cases without clinical disease, one reaches a maximum figure of 11 per cent of all O-A and O-B incompatible pregnancies. Reepmaker[14] believes that "typical" hemolytic disease is due to ABO iso-immunization in 5 to 10 per cent of the cases, but that if icterus praecox were included, the number might exceed that of Rh hemolytic disease.

Clinical Manifestations

The classic picture is dominated by the symptom of jaundice, anemia playing a far less conspicuous role than it does in Rh hemolytic disease. Characteristically, icterus makes its appearance in the first 24 hours after birth and often within the first few hours, despite the fact that the infant may otherwise appear healthy and vigorous. The icterus may be the only clinical finding and may be transient, disappearing as early as the second or third day. It may persist through the period of physiological icterus or may last into the second week of life. Its intensity is highly variable. If one regards bilirubin levels above 12.5 mg. at any time in the newborn period as pathologic, many infants have a more or less marked hyperbilirubinemia. The levels may be as high and the clinical icterus as intense as in Rh hemolytic disease.

Pallor is uncommon in the first few days of life because the anemia is usually mild and often minimal in ABO hemolytic disease. Untreated, the anemia may become more marked, and occasionally one sees a severely anemic infant in the second or third week of life. Occasionally anemia is pronounced at the time of delivery and the infant presents a picture of marked pallor, shock and even cardiac failure as in typical Rh erythroblastosis. Frank hydrops has been reported in ABO hemolytic disease, but appears to be extremely uncommon.

The spleen and liver may be moderately or slightly enlarged. Purpura has not been encountered in our series of 220 consecutive cases.

The infants are usually alert, at least at first. Definite lethargy should be a warning that a bilirubin encephalopathy may be in the offing and is usually associated with high bilirubin levels in the serum. Spasticity, opisthotonos, loss of Moro reflex, vomiting, ocular signs, high-pitched shrill cry, irregular breathing and convulsions are, of course, to be regarded as evidence of kernicterus unless there is another obvious explanation for these symptoms. Kernicterus does occur in ABO hemolytic disease and is, in fact, the chief concern of the therapist, regardless of how "mild" the hemolytic process may appear to be.

History

In about 40 to 50 per cent of the cases the first-born child is affected. The likelihood is great that the first incompatible offspring is often affected and that a negative history of jaundice in previous children of the same blood group is unreliable because of the mildness of the symptoms. A history of stillbirths or of hemolytic disease of increasing severity is decidedly unusual. We have been impressed with the uniformity of the clinical picture in successive infants born to the same mother in a number of families we have been able to study.

The Blood Picture

The most characteristic finding in ABO hemolytic disease is spherocytosis[5] (see Fig. 9). This finding is particularly striking in the blood of the newborn, which is normally macrocytic. Spherocytes may be abundant or sparse. There is reason to believe that the number may rapidly decrease, so that after the first few days the diagnosis becomes increasingly difficult. In examining the blood film it is important to look for spherocytes only in areas in which excessive thicknesses or thinness of the smear has not produced artifacts. Particularly at the edges of an ordinary slide preparation flattening and rapid drying can simulate spherocytosis.

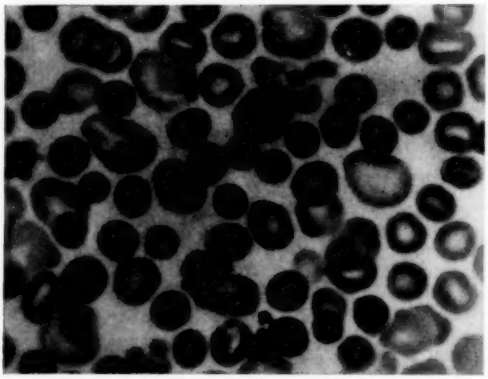

Fig. 9. Spherocytosis in ABO hemolytic disease.

Along with the presence of spherocytes an increase in the osmotic fragility can usually be demonstrated.[3] For routine diagnostic use this method is somewhat unsuitable because of the technical difficulties and the time it consumes.

The hemoglobin and the red cell count may be entirely within normal limits. Often they are slightly or moderately lowered. In the majority of cases observed by us the hemoglobin levels were above 10 gm., usually in the range of 12 to 13 gm. per 100 ml. when capillary blood from the heel was examined.

Even when the hemoglobin is in the normal range, examination of the smear and determination of the reticulocyte count usually indicate a compensated hemolytic process. There is polychromasia in excess of that seen in the blood film of the normal newborn infant, and reticulocytosis up to 25 or 30 per cent is not uncommon.

Normoblastemia is somewhat less common and, when present, is usually far less marked than in Rh hemolytic disease. By the same token

the total (uncorrected) white blood cell count is at best only moderately elevated in most cases. We have not seen thrombopenia such as occurs in severe Rh erythroblastosis.

Other Laboratory Findings

The elevation of the serum bilirubin level is probably the most significant finding. The serum bilirubin is either exclusively or predominantly of the so-called indirect variety, but occasionally, especially when the total pigment is greatly elevated or when severe jaundice has existed for some time, an elevation of the "direct" bilirubin may also occur and the test for "bile" in the urine may become positive. We have not seen loss of coloration of the stools.

The bilirubin levels in 220 cases of hemolytic disease studied by us ranged from 2.8 to 65 mg. per 100 ml. The interpretation of these levels depends in part on the time of the determination. On the first day of life a level of 10 mg. suggests that far higher levels may be reached in the next few days, whereas the same level on the second or third day indicates a more benign course.

Serologic Findings. 1. The basis of the serologic diagnosis is the demonstration of incompatibility between mother and child in the ABO blood groups and the exclusion of Rh or other blood factor sensitization. In most cases the mother is group O and the infant group A or B. In our series only two of 207 mothers were not group O. These two women were blood group A_2. The incidence of less than 1 per cent of non-O mothers makes it unlikely that hemolytic disease is due to anti-A or anti-B when the mother is not group O.

2. The subgroup of the group A infants is of interest. The maternal antibody in cases of O-A hemolytic disease tends to have anti-A_1 specificity. It might therefore be expected that such antibody is effective only in the A_1 infants. The development of the subgroups of A is, however, incomplete at the time of birth. Witebsky and Engasser[24] showed that many infants who subsequently prove to belong to subgroup A_1 at birth give the reactions of A_2. This observation was confirmed in our laboratory.[29] In our series of 142 infants with O-A hemoglobin disease, 82 were subgroup A_1 and 54 A_2 at birth. In 14 cases it was possible to retest the apparent A_2 infants at a later date. In each case the infant was found to be A_1.

In order to obtain further information on this subject the subgroups of A of the fathers were investigated. In order for the infant of an O mother to belong to subgroup A_1, the father must be A_1 or A_1B and belong to one of the following genotypes: A_1A_1, A_1A_2, A_1O or A_1B. An A_2 father, whose genotype can be only A_2A_2, A_2O or A_2B, cannot have A_1 children from a group O mother. Among 82 fathers of A in-

fants whose subgroup was known, 73 proved to be A_1 and nine A_1B. There were no A_2 or A_2B fathers. The expectation would be for 20 per cent A_2 of group A individuals in a random sample. The significance of this discrepancy was strengthened by the observation that among the 37 fathers of infants with apparent A_2 reactions at birth whose subgroup was known, not one belonged to A_2 or A_2B.[26] It appears, therefore, that only or almost only infants whose true subgroup is A_1 are susceptible to O-A hemolytic disease. This is in keeping with the greater reactivity of A_1 erythrocytes as well as with the specificity of the maternal antibody.

The determination of the infants' subgroup being unreliable at birth, these facts unfortunately are of little help from a practical viewpoint. However, if the father of an A infant can be shown to belong to subgroup A_2, the diagnosis of O-A hemolytic disease becomes unlikely. For blood group B subgroups are not clearly established and the question has no practical application.

3. The direct Coombs test should always be done. If it is strongly positive, Rh or some other factor is likelier than O-A or O-B hemolytic disease. In our series the test was positive in 66 of 212 cases, or 31.1 per cent. Only in 13 cases, or 6.1 per cent, was the direct Coombs test grossly positive, and a reaction read as ++ was obtained in only two cases. Usually the positive reading is based on microscopic examination.

The procedure for the direct Coombs test is as follows:

The patient's erythrocytes are suspended in an excess of cold saline and washed 3 times. After the last washing the button of cells is resuspended in sufficient saline to make an approximate 2 per cent suspension in a serological test tube. Adult group O cells are similarly processed for control purposes. Depending on the manufacturer's instructions, 2 drops of each cell suspension are mixed with one or 2 drops of the antiglobulin serum and centrifuged either immediately or after 5 minutes at room temperature, at a speed of 800 to 1000 revolutions a minute for one minute. The tube containing the control cells and the tube with the patients' cells are held simultaneously over a concave mirror and read grossly by gently tapping or flicking both tubes. If the gross reading is negative, the contents of the tubes are poured into the wells of a deep-well slide and immediately read under a low power microscope. Any clumping observed in the patients' cells in the absence of agglutination in the control cells is read as ±.

4. Whether the direct Coombs test is positive or negative, the most informative test in our hands has been the demonstration of free antibody in the infant's serum of agglutinating adult erythrocytes of the same blood group as the patient. As mentioned before, the normal group A infant of an O mother has no free anti-A in its serum, though anti-B may be present. Conversely, the B infants of O mothers show no free anti-B. The demonstration of the homologous antibody may therefore be regarded as a pathological finding indicating excess maternal antibody.[25] It is usually positive in O-A and O-B hemolytic disease. In our series the demonstration was successful in 202 of 210 cases studied, or slightly better than 96 per cent.[26]

Rarely, free antibody can be found in the serum of apparently normal infants. In our experience most of these infants belonged to blood group A_2, in which the low reactivity of the fetal erythrocytes may well explain the lack of disease in the presence of free homologous antibody.

The procedure for the demonstration of free homologous antibody is as follows:

The infant's serum is inactivated at 56° C. for 30 minutes. Meanwhile adult erythrocytes of group A_1 or B, depending on the patient's own blood group, are washed and suspended in approximately 2 per cent concentration in 0.9 per cent saline solution. A control suspension of adult O cells is prepared in a similar manner. Two drops of serum are added to 2 drops of each of the A or B and to the O control cell suspensions, and each mixture is incubated in a 37° C. water bath for a half hour. The tubes are then centrifuged at 2500 revolutions a minute for 2 minutes. Gross agglutination is observed while simultaneously holding and tapping the A or B and the O control cell suspension. The simultaneous reading of the O control suspension is important because the agglutination of the A or B cells in saline is often weak and the clumps are easily shaken out. The difference in the behavior between the test cells and the control cells must therefore be carefully observed. If no agglutination of the A or B cells is noted in saline medium, the indirect Coombs test is performed by triple washing of the cell suspensions and adding antiglobulin serum and proceeding as in the direct Coombs test.

5. *Tests with the Maternal Serum.* Since by definition antibodies antagonistic to the fetal blood group are always present in heterospecific pregnancy, the mere demonstration of α or β agglutinins is of little help in the diagnosis of ABO hemolytic disease. The various tests designed to demonstrate "immune" anti-A or anti-B are somewhat cumbersome and not apt to be helpful in the diagnosis when the infant is born. The prenatal diagnosis is even more unreliable because, as mentioned in the theoretical section, the correlation with clinical hemolytic disease is far from satisfactory. Since the infants with ABO hemolytic disease are almost always liveborn and usually in good condition at the time of delivery, a prenatal diagnosis is not as essential here as it is in Rh hemolytic disease, provided icteric infants are promptly investigated.

The maternal serum should always be tested against O Rh-positive and O Rh-negative cells, and preferably against a panel of group O erythrocytes representative of such factors as C^w, Kell, Duffy, S, and others, in order to rule out Rh and rare factor hemolytic disease. These tests require the use of the indirect Coombs technique. The titration of anti-A or anti-B in saline solution may be of some help if the titer proves to be extraordinarily high. In our experience titers of less than 1024 against A and 512 against B are of no diagnostic value.

Of the various techniques for the demonstration of "immune" anti-A or anti-B the neutralization test of Witebsky[23] and the hemolysin test have found the widest application. The neutralization test in our hands

has given the best correlation with clinical hemolytic disease among the techniques tried in our laboratory.

The test in essence consists of 2 parts as originally described by Witebsky. The first part is a regular titration procedure in saline medium, using the test tube method. In the second part the serum is mixed 1:1 with soluble A and B substance (Sharp & Dohme, Inc.). Twofold dilutions are made of the mixture, and each dilution is tested against a suspension of A or B adult erythrocytes by means of the indirect Coombs test. The original method of Witebsky called, instead of the Coombs test, for titration of the serum-soluble mixture in AB serum. The results of the two methods are similar.

The hemolysin test of Mollison[12] depends on the presence of complement. It can be done with fresh maternal serum, but this is not always feasible. If it is necessary to store the serum, a source of fresh complement must be added at the time the test is made. Fresh human AB serum is preferable to lyophilized guinea pig complement.

Test cells, adult A_1, A_2, B and O erythrocytes and, whenever possible, the infant's own red corpuscles are washed in saline 3 times. After the third washing the supernatant is removed as completely as possible and the packed red cells are carefully measured to make an accurate 5 per cent cell suspension in fresh AB serum. Then 0.2 cc. of each cell suspension is added to 0.2 ml. of maternal serum. These mixtures are well agitated and then allowed to stand in a 37° C. water bath for 2 hours, the tubes being shaken several times during this period. At the end of 2 hours the tubes are centrifuged for one minute at 2500 revolutions a minute and read grossly for hemolysins. The results are graded negative, one to 4 plus, by comparing each tube against the control O cells. A 4 plus reading has complete hemolysis with no cells at the bottom.

TABLE 11. *History and Clinical Findings in ABO Hemolytic Disease*

HISTORY	CLINICAL FINDINGS
Often 1st pregnancy or 1st heterospecific pregnancy	Early onset of jaundice
History of previous siblings unreliable	Few other symptoms
No stillbirths	Anemia mild
Common in Negro and white	Splenomegaly slight
	Usually early recovery
	Kernicterus may occur

TABLE 12. *Laboratory Findings in ABO Hemolytic Disease*

Rh iso-immunization ruled out	Saline agglutination or indirect Coombs test with adult A or B (homologous) cells positive
Mother nearly always group O	Anti-A usually = anti-A_1 all infants presumed A_1
Maternal anti-A and/or B often high	Anemia usually mild
Maternal immune anti-A and/or B usually present (inconstant)	Reticulocytosis, spherocytosis, indirect bilirubin elevated
Infant's direct Coombs test usually negative or (40%) weakly positive	

In our experience the results of the Witebsky technique correlated best with the clinical findings of ABO hemolytic disease. When a titer of 32 or more was obtained with the neutralized serum, a correlation of 86 to 87 per cent was obtained. Lower titers were frequently found in apparently normal cases and did not appear to be significant. *In the*

nearly 15 per cent of cases with a negative test the clinical hematologic picture was in no way different from that in cases with a positive test.

The presence of hemolysins is a common finding in group O serums. There are gradations from one to 4 plus. The readings are influenced by the volume of cells used and, in our experience and that of Hubinont,* by the source of complement, more positive results being obtained when the maternal serum itself is used fresh than when human complement is added later. Because some hemolysis is common, Mollison proposes to regard only the hemolysis of the fetal erythrocytes as significant in confirming the diagnosis of anti-A or anti-B hemolytic disease. In our experience the correlation has not been satisfactory.

Tables 11 and 12 summarize the salient clinical and laboratory findings in ABO hemolytic disease.

Management

The majority of infants with ABO hemolytic disease probably require no therapy, but should be watched closely. The danger lies in the development of excessive hyperbilirubinemia with consequent development of kernicterus. This is virtually the only danger to which these patients are exposed, except in the rare instances of marked anemia and cardiac failure. The treatment of both these complications is essentially the treatment of Rh hemolytic disease, which is discussed by Wheeler and Ambuel (p. 383), whose views we endorse. The point to be stressed is the frequent dissociation between the intensity of the hemolytic process, which may be mild or even completely compensated, and that of the bilirubinemia and jaundice, which may be severe. Since the danger seems to be related to the bilirubinemia, mildness or severity must be judged in terms of this finding rather than on the degree of anemia or other clinical findings.

At present the only effective means of preventing or combating hyperbilirubinemia is exchange transfusion. The indication as to when and as to whether an exchange transfusion has to be done in ABO hemolytic disease, in our experience, depends almost solely on the actual or anticipated degree of hyperbilirubinemia. One cannot set an arbitrary level of the serum bilirubin because, as mentioned earlier, the time factor must be taken into consideration. By and large, in hemolytic disease the higher the initial level, the more rapid the rise and the higher the peak level reached in the next few days in untreated cases. This statement is somewhat more true of Rh than of ABO hemolytic disease. It is our belief that one has somewhat more leeway in the latter condition if one has the laboratory facilities for the frequent de-

* P. O. Hubinont: Personal communication.

termination of the bilirubin level. One can frequently wait and observe developments before deciding to do an exchange transfusion.

In our own institution we are inclined to individualize, taking into account the bilirubin level, the age at onset of jaundice, the age of the child and its general condition. As a rough guide we have found the following approximations useful: If the bilirubin is

less than 10 mg. per 100 ml. in the first 24 hours or
less than 14 mg. per 100 ml. in the second 24 hours or
less than 17 mg. per 100 ml. in the third or fourth day

in a full term infant, we follow an expectant course, observing the baby clinically and with bilirubins determined every four to eight hours. If the bilirubin is higher than the above values at the times indicated, we tend to perform an exchange transfusion. If, however, one is dealing with a borderline value—i.e., the child in clinically good condition and a full term infant—we frequently wait a few hours more. If by this time there has been no further rise in the bilirubin or only a slight increase, we are inclined to temporize further; otherwise the exchange transfusion is now performed. We do not regard an exchange transfusion as an entirely harmless procedure even in experienced hands and therefore like to follow fairly clear-cut indications. If, however, there is a reasonable doubt, if the infant is significantly premature or there is no opportunity for close supervision, exchange transfusion on the basis of the values given above should be fairly rigidly adhered to. In following this policy we are aware that we are deviating from the assumption that exchange transfusion in hemolytic disease is the more efficient, the earlier it can be carried out. However, in ABO hemolytic disease the bilirubin levels often rise more slowly, more often drop spontaneously and generally are more unpredictable than in Rh hemolytic disease. If one followed, slavishly, fixed values as indications for exchange transfusion (except in the very high ranges), one would no doubt often perform the procedure unnecessarily. Moreover, our experience has taught us that in ABO hemolytic disease the indication for a second exchange transfusion rarely arises even though the original procedure was not carried out until the second or third day. However, at times it does become necessary to perform a second exchange transfusion on the basis of a bilirubin level rising as high as or higher than the original value, and in rare instances even a third and fourth exchange transfusion has to be carried out on this basis. In following such a policy we have seen no instances of kernicterus except in infants admitted at several days of age and already showing severe jaundice and signs of encephalopathy.

The type of blood to be used in exchange transfusion in ABO hemolytic disease is not securely established by controlled data. On theoretical grounds we have used group O blood of the same Rh type

as that of the infant. For equally theoretical reasons we have insisted that this blood be naturally low in titer with respect to its α and β iso-agglutinins. We have not used high-titered O blood or O blood of unknown agglutinin content, to which soluble A and B substances have been added. For practical reasons we insist that the blood should be as fresh as possible in order to minimize both in vitro and in vivo hemolysins. Bank blood over six days of age is not acceptable. Blood less than three days old is preferable. When exchange transfusion is not indicated, but some degree of anemia exists, a simple transfusion of whole blood or sedimented red blood cells is given. We attempt to maintain a hemoglobin level above 12 gm. per 100 ml.

Course and Prognosis

As stated before, kernicterus is virtually the sole danger to infants with ABO hemolytic disease. The true incidence of this complication depends on the frequency with which the diagnosis of ABO hemolytic disease is made. If one includes the cases of so-called icterus praecox, the incidence of kernicterus is probably low. In a series severe enough clinically to be transferred to a Children's Hospital with a more or less typical picture of hemolytic disease, the incidence was between 10 and 15 per cent before the institution of a regimen such as outlined above. When kernicterus is prevented, the mortality is virtually zero, allowing for the rare hazards connected with exchange transfusion itself.

It is important for pediatrician, obstetrician and family alike to understand this fact and to realize that the prognosis for future children of susceptible blood group is essentially the same. We know of no indications for obstetrical intervention, sterilization, early termination of pregnancy, and the like, in cases in which a woman has produced a liveborn infant with hemolytic disease. The literature contains a few instances of stillbirth attributed to anti-A and anti-B in women[5, 7] with, as a rule, extraordinarily high antibody titers. Such cases are more likely to come to the attention of the serologist than of the pediatrician.

Differential Diagnosis

All the possible causes of jaundice in the newborn period must be considered in the differential diagnosis. Once O-A, O-B incompatibility has been established, it is still necessary to rule out physiological jaundice, Rh hemolytic disease or maternal iso-immunization against C^w, Duffy, S, and others, congenital hemolytic anemias, hepatitis and other viral infections, congenital syphilis, to name the principal sources of confusion.

The differentiation from *physiological jaundice* rests chiefly on the

demonstration of a hemolytic process in patients with ABO hemolytic disease, viz., reticulocytosis and spherocytosis. Physiological jaundice, even though the bilirubin levels may reach an excessive and perhaps a dangerous height, is not observed in the first 24 hours of life. Rh hemolytic disease is readily ruled out by the proper use of the serologic tests for that condition. When mother and infant are incompatible in O-A-B blood system, the likelihood of Rh iso-immunization of the mother is much smaller than in those types of pregnancy in which there is no maternal iso-agglutinin against the major blood group of the fetus. For reasons not entirely clear spherocytosis, which is characteristic of ABO hemolytic disease, is seldom seen in *Rh erythroblastosis*. We have seen it in a few instances of combined Rh and ABO hemolytic disease. In Rh hemolytic disease, generally, anemia, normoblastemia and hepato-splenomegaly are generally more marked and the child is more apt to appear clinically ill.

Congenital hemolytic anemias can occasionally give rise to considerable confusion if the hemolytic process happens to be active at birth. We have recently seen a number of such instances, particularly cases of congenital spherocytosis in which both the blood picture and the clinical picture resembled, at birth, hemolytic disease of the newborn. In such cases, if the blood groups of mother and fetus are incompatible, the diagnosis may present considerable difficulties. The negative direct and indirect Coombs tests of the infant's blood in the presence of spherocytes should direct one's attention to this possibility. Often, though not invariably, a family history in such cases is positive, and the study of the blood picture and erythrocyte fragility of both parents should be helpful. We have seen cases in which both ABO hemolytic disease and congenital spherocytosis could be diagnosed. The exact diagnosis cannot always be made at the time of birth, and only the presence of a hemolytic process may be established. In this situation one should not be too concerned with making an exact etiological diagnosis, but treat the infant according to the indications given above. It does not seem to matter whether one removes congenitally defective red corpuscles or cells damaged by antibodies. If the hemolytic process leads to severe hyperbilirubinemia, kernicterus can occur in both situations and can be prevented in both instances by the proper use of exchange transfusion.

Neonatal hepatitis presumed to be due to a viral agent such as that of homologous serum jaundice or cytomegalic inclusion disease in the presence of O-A or O-B incompatibility may present diagnostic difficulties, particularly because they may be associated with a hemolytic process. The absence of positive serologic findings and of spherocytosis, generally speaking, militates against ABO hemolytic disease, while the presence of an elevated direct-reacting bilirubin at an early stage, dis-

turbed liver function tests and a clinically ill infant are points in favor of hepatitis. The diagnosis of congenital syphilis usually presents no difficulties.

SUMMARY

Heterospecific pregnancy produces a variety of effects ranging from a completely normal state to a condition in which subclinical effects on fetal erythrocytes and erythrocyte survival can be demonstrated, to mild clinical hemolytic disease apparent as icterus praecox, and to more marked degrees of clinical involvement resembling an occasionally equally classical Rh hemolytic disease. The demarcation between clinically normal and frankly pathological states is difficult and uncertain.

The chief manifestation of clinically recognizable ABO hemolytic disease is early intense jaundice. The accompanying anemia may be minimal or fully compensated, mild or of moderate severity. It is rarely marked. The main hematologic findings are spherocytosis and reticulocytosis.

The serologic findings include incompatibility in the ABO system, with the mother usually group O. The maternal antibody pattern is variable and at present does not correlate satisfactorily with the clinical-hematologic findings in the infants. Tests involving the maternal serum are, moreover, too cumbersome for immediate postnatal diagnosis.

The serologic diagnosis at present rests chiefly on the demonstration of free antibody affecting the infants' blood group in the infants' own serum either by direct saline agglutinin or by the indirect globulin technique.

The chief danger to infants with ABO hemolytic disease arises from the possible development of marked hyperbilirubinemia attended by kernicterus. Treatment and prevention of this complication are the main aims of therapy. The indications for exchange transfusion based on the preceding considerations are outlined.

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AUTO-IMMUNE HEMOLYTIC ANEMIAS

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Acquired hemolytic anemias constitute a heterogeneous group of diseases which occur frequently in infants and children. One feature common to the entire group, irrespective of etiology, is destruction of the patient's red cells by the action of factors *extrinsic* to the red cells. This group is distinguished from the hemolytic disorders resulting from rapid destruction of *intrinsically* defective erythrocytes. It is the purpose of this discussion to characterize one particular variety of acquired hemolytic disease in which plasma proteins formed by the patient react with his own erythrocytes, leading to their premature destruction. Because these proteins behave like antibodies and react with a tissue (red cells) of the individuals who form them, they are called "auto-antibodies." The hemolytic disorders associated with the presence of demonstrable auto-antibodies form a distinctive subgroup of the acquired hemolytic anemias, and they are collectively referred to as auto-immune hemolytic anemias.

One particular auto-immune disease with singular characteristics is best known as paroxysmal cold hemoglobinuria (PCH) and will be discussed separately. The designation AHD (auto-immune hemolytic disease) is applied here to the auto-immune hemolytic disorders exclusive of paroxysmal cold hemoglobinuria.

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It is now possible for the clinician to establish the correct diagnosis in most cases of auto-immune hemolytic disease. Widespread use of recently developed laboratory procedures for the detection of antibodies[14] is largely responsible for the recognition and proper treatment of increasing numbers of cases of auto-immune hemolytic disease in pediatric patients. The antiglobulin (Coombs) test has been especially valuable in case detection and is now a standard procedure in most clinical laboratories.

GENERAL CONSIDERATIONS REGARDING HEMOLYTIC ANEMIAS

The destruction of senile erythrocytes is a normal process and is properly referred to as hemolysis. Human red cells normally have a life span (survival) of approximately 120 days after release from the bone marrow. *Whenever the survival of red cells is shortened, a hemolytic disorder exists.* This is equivalent to saying that if the rate of red cell destruction is increased, a hemolytic disease exists. Under physiological conditions this rate is slightly less than 1 per cent per day, and any measurable increase represents pathological hemolysis. Direct determination of red cell survival is not ordinarily carried out for diagnostic purposes. It is well to remember, nevertheless, that abnormal clinical and laboratory findings in patients with hemolytic diseases are the consequences of decreased red cell survival.

Normally the rate of red cell destruction is balanced by a corresponding rate of red cell production.* In the presence of a moderate increase in the rate of red cell destruction (e.g., twice normal rate, red cell life span reduced to 60 days), the bone marrow usually compensates by increasing the production of new red cells. When anemia is absent, the process exemplifies *compensated hemolytic disease.* Only when increases in the rate of red cell destruction exceed the ability of the body to increase its rate of production does anemia result (*decompensated hemolytic disease*). The limits of the ability to compensate for accelerated red cell destruction have been estimated for adults,[8, 24] but are not established for infants and children. It seems likely, however, that this ability to compensate is least at birth and increases progressively throughout childhood. Thus an increased rate of red cell destruction is usually reflected in an increased rate of red cell regeneration. The manifestations of accelerated regeneration which are clinically important are reticulocytosis and normoblastemia in the peripheral blood, and erythroid hyperplasia in the bone marrow. Spherocytosis is a variable and nonspecific finding in relation to hemolytic diseases, being present in some and not in others. In disorders in which it does occur

* The only physiological exception to this is during the first few months of life when erythropoiesis in the marrow is relatively inactive.

it may be either a manifestation of the production of intrinsically defective erythrocytes, or the result of damage to normal cells by extrinsic factors.

Hemoglobin released by the destruction of red cells is normally converted to bilirubin and excreted by the liver into the intestine. Jaundice may be absent in patients with active hemolytic disease if the liver is able to increase the rate of pigment excretion,* but the fecal excretion of urobilinogen is increased. A portion of the pigment is reabsorbed from the intestine and excreted in the urine, and it is not uncommon for the urinary urobilinogen excretion to be increased when hemoglobin breakdown is accelerated.[9]

TABLE 13. *Indications of Increased Hemolysis*

EVIDENCE OF DESTRUCTION	EVIDENCE OF REGENERATION
Hemoglobinemia	Reticulocytosis
Hemoglobinuria	Normoblastemia
Hyperbilirubinemia (indirect-reacting bilirubin)	Erythroid hyperplasia in bone marrow
Jaundice	
Increased pigment excretion (fecal and urinary urobilinogen)	
Anemia (in decompensated hemolytic state)	

If intravascular hemolysis occurs, hemoglobinemia may become apparent. If the plasma concentration of hemoglobin exceeds the renal threshold for this substance, hemoglobinuria occurs. (It is important to distinguish carefully between hemoglobinuria and hematuria.[26]) Hematin released from hemoglobin is linked to serum albumin to form methemalbumin, which may impart a brownish tinge to the serum, and usually reflects intravascular or exceedingly rapid hemolysis.[22]

Table 13 summarizes the indications of increased hemolysis which are of practical value.

NOMENCLATURE AND CLASSIFICATION

The designation "auto-immune hemolytic disease" (AHD) is preferred to names using the words "anemia" or "jaundice," since either

* This reserve capacity to excrete bilirubin seems minimal at birth, but increases during early infancy. Infants in the first week of life may thus be able to effect hematological compensation for increased red cell destruction and have no anemia, but be unable to excrete the extra bilirubin produced and become deeply jaundiced. This may result in brain damage (kernicterus), discussed in other papers in this volume. Though no cases of kernicterus associated with auto-immune hemolytic disease appear to have been reported, the possibility of this complication exists.

or both may be absent at times. Numerous classifications of auto-immune hemolytic disease have been made.[16, 18] Most important for clinical purposes is an appreciation by the physician that auto-immune hemolytic disease may be either idiopathic (etiology unknown) or symptomatic (associated with other disorders). Table 14 lists the disorders with which symptomatic cases have been (or may be expected to be) associated in childhood.

TABLE 14. *Classification of Auto-immune Hemolytic Disease*

TYPE OF AUTO-IMMUNE HEMOLYTIC DISEASE	ASSOCIATED DISEASE
Idiopathic	None
Symptomatic	(1) Collagen disorders[14, 17, 31, 44, 54]
	Disseminated lupus erythematosus
	Periarteritis nodosa
	Rheumatoid arthritis
	Ulcerative colitis
	(2) Granulomatous disease[38]
	Boeck's sarcoid
	(3) Lymphoma[14, 44]
	Hodgkin's disease
	Reticulum cell sarcoma
	Lymphosarcoma
	(4) Infections[1, 14, 32, 41]
	Hepatitis
	Infectious mononucleosis
	Typhoid fever
	Viral pneumonia
	(5) Drug idiosyncrasy with hemolytic anemia[27, 48]

The course of either type may be transient or chronic. It is usually not possible at the time the diagnosis is made to predict which course the disease in any given patient will follow.

CLINICAL FEATURES

None of the clinical features serves to distinguish auto-immune hemolytic disease from other forms of acquired hemolytic disease. The disease has affected infants as early as two weeks of age[1] and occurs at any age throughout childhood in patients of either sex. No genetic influence has been recognized in auto-immune hemolytic disease, and familial occurrence has not been reported. There is apparently neither racial predilection nor immunity.

The onset of the disease may be abrupt or insidious. The signs and symptoms may be related to anemia, accelerated hemolysis or an underlying disease (as in lupus and others). The symptoms of headache and palpitation, frequently referable to severe anemia in adults, are seldom

encountered in pediatric practice. Irritability, anorexia, low grade fever and either drowsiness or fretfulness are frequent findings in infants with severe anemia. Older children may complain of fatigue and display diminished exercise tolerance. Pallor (of mucous membranes) may be striking, but icterus, if present, is usually mild. Although these patients *frequently* present themselves with jaundice, anorexia and dark urine, differentiation from hepatitis is readily made. It is probable that splenomegaly is almost always present, but in children in whom anemia develops with great rapidity the spleen may not be palpable initially. Massive splenomegaly is uncommon in auto-immune hemolytic disease. In a proved case of auto-immune hemolytic disease massive splenomegaly should suggest the presence of an associated or underlying disease.

HEMATOLOGICAL FINDINGS

Presence or absence of anemia is determined by the rate of red cell destruction and the degree of compensatory regeneration. Accordingly, anemia may be absent, or of variable severity if present. Spherocytosis is usually, but not invariably, observed during periods of active hemolysis. It is lacking during relatively quiescent periods in patients with chronic or recurrent disease. Reticulocytosis (and therefore polychromatophilia) is an important feature, and is usually most pronounced when anemia is most profound. Microcytosis due to sphering and macrocytosis due to the increased number of reticulocytes account for the anisocytosis observed in blood smears. The association of small, densely staining microspherocytes with polychromatophilic macrocytes is characteristic of active hemolytic disease, *but is not by itself diagnostic of auto-immune hemolytic disease*. During periods of accelerated hemolysis nucleated red cells enter the peripheral blood in variable numbers and may be especially numerous if hemolysis persists or recurs after splenectomy. These features are well shown in Figure 10.

Although reticulocytes are usually increased in proportion to the severity of the hemolytic process, reticulocytopenia of variable degree may be encountered in auto-immune hemolytic disease.[10] Temporary cessation of erythropoiesis has been observed; in the presence of rapid hemolysis a deficiency in production of red cells leads to sudden and profound increase in anemia.[5, 20, 37]

Auto-agglutination of the erythrocytes is frequently observed in samples of peripheral blood. Inasmuch as the patient's sensitized cells may agglutinate in any human serum, blood grouping may be difficult. Indeed, difficulties encountered in blood grouping and cross-matching may provide the first clue to the nature of the process in patients with

hemolytic anemia. It has been emphasized that these phenomena must be distinguished from rouleau formation which is present in a variety of disease states.[50]

Osmotic fragility of the red cells is increased only during periods of rapid hemolysis. During quiescence of the disease spherocytes are absent, and osmotic fragility is normal. Measurements of osmotic fragility of the red cells after sterile incubation at body temperature[50] and measurements of spontaneous autohemolysis[52] in vitro frequently aid in differentiating auto-immune hemolytic disease from other hemolytic disorders with which it might be confused.

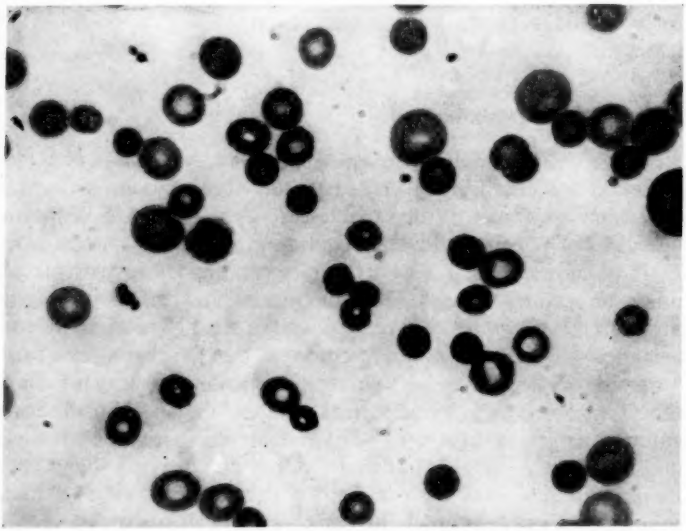

Fig. 10.

Leukocyte counts may be greatly increased (up to 30,000 to 50,000 per cu. mm.) during active hemolytic episodes. As a rule, mature neutrophils account for most of the increase, but a moderate "shift to the left" is common. In patients who have undergone splenectomy both the leukocytosis and the release of immature cells are accentuated. Occasionally erythrophagocytosis may be seen in blood smears, or in smears prepared from the buffy coat of oxalated blood.[54] Neutropenia is occasionally found, but is not characteristic of the disease.

Platelets are usually present in normal numbers, although in some patients thrombocytopenia may be a prominent feature of the disease. In fact, the coexistence of anemia and thrombocytopenia led Evans[21] to suggest that both were manifestations of activity of auto-antibodies.

Swisher[40] has recently observed a patient who had undergone splenectomy in childhood during a bout of idiopathic thrombocytopenic purpura, and subsequently had auto-immune hemolytic disease during early adulthood. The youngest reported patient affected with auto-immune hemolytic disease and thrombocytopenia was seven weeks old.[23]

SEROLOGIC FINDINGS

Serologic studies form the usual basis for distinguishing auto-immune hemolytic disease from other hemolytic anemias. Though there is still difference of opinion as to whether the erythrocyte-sensitizing globulins in auto-immune hemolytic disease are really antibodies, for the sake of simplicity in this discussion they will be referred to as antibodies. Many analogies exist between the auto-antibodies in auto-immune hemolytic disease and typical blood group antibodies. In fact, some recent examples of auto-antibodies with blood-group specificity (in particular anti-e and other Rh antibodies) have been demonstrated.[15, 42, 43, 45]

Most auto-antibodies in patients with auto-immune hemolytic disease behave as incomplete antibodies, and are with few exceptions active at body temperature in childhood cases. Specialized serologic techniques may be required for the demonstration of auto-antibodies, and espe cially for the demonstration in vitro of their hemolytic properties.[12] Antibody may be demonstrated free in the patient's serum, adsorbed to the patient's erythrocytes, or in eluates prepared from the patient's red cells or red cell stroma. Serologic behavior of the antibodies may vary greatly from case to case, and in the same patient at different times in the disease. Other serologic abnormalities may be encountered, such as the presence of sheep cell agglutinins, falsely positive Wassermann reaction, and alterations in serum complement.

Initial serologic tests are best directed at the detection of auto-antibodies adsorbed to the patient's erythrocytes. The antiglobulin (Coombs) test has served as a sensitive test for erythrocyte autosensitization in auto-immune hemolytic disease since 1946,[4] and should be done whenever a hemolytic process is suspected. If the test is properly performed, agglutination of the patient's red cells by antiglobulin serum indicates that the cells are coated by auto-antibodies. Tests must be made with appropriate dilutions of the reagent in order to avoid falsely negative reactions, and it is also well known that antiglobulin reagents differ in their ability to detect erythrocyte sensitization.[11, 13, 33] Hence well equipped laboratories usually use more than one antiglobulin serum if red cell sensitization is difficult to demonstrate in a patient in whom auto-immune hemolytic disease is strongly suspected.

The erythrocytes of patients with auto-immune hemolytic disease are

frequently agglutinated by normal human serum or in serum-albumin mixtures.[2] A simple and promising technique recently introduced utilizes polyvinyl pyrrolidone (PVP) to detect red cell sensitization.[29] A battery of such serologic procedures may be required to demonstrate erythrocyte sensitization in atypical cases.

Auto-antibodies in the patients' serums are frequently capable of sensitizing normal erythrocytes for the antiglobulin reaction, or causing agglutination of enzyme-treated normal red cells. Temperature, pH and the presence or absence of complement may influence the results of tests for serum auto-antibodies.[14] Fluctuations in the concentration of antibody detected do not always parallel the fluctuations in hemolytic activity of the disease.[16, 51]

The nature of the stimulus to the formation of auto-antibodies is not recognized in most cases. Recent important studies have demonstrated that specific inciting (chemical) agents can be identified in the immune type of red cell destruction in some cases of auto-immune hemolytic disease.[27] Such work has important implications for improved understanding of the etiology and pathogenesis of diseases associated with auto-immunization, of which auto-immune hemolytic disease is but one example.[18]

FATE OF TRANSFUSED NORMAL BLOOD

In patients with auto-immune hemolytic disease transfused normal erythrocytes are susceptible to the damaging action of the auto-antibodies. The donated red cells become sensitized, and there is evidence that they may be destroyed even more rapidly than the patient's own red cells.[25] When spherocytosis of the patient's cells is present, transfused cells may become spherocytic, and are then as readily trapped in the patient's spleen as are his own erythrocytes.[53]

ROLE OF THE SPLEEN

In auto-immune hemolytic disease the spleen probably functions as a site of antibody formation and as a site for red cell destruction. It is now thought that the spleen retains and destroys sensitized red cells by (*a*) promoting agglutination and sequestration within the splenic circulation,[28] (*b*) trapping spherocytic cells,[46, 49] and (*c*) acting as a site for erythrophagocytosis.[39] Except for erythrophagocytosis, the precise mechanisms by which cells are damaged or destroyed in the spleen remain conjectural. Since the course of auto-immune hemolytic disease is little affected by splenectomy in some patients, it is obvious that the spleen is only one of many tissues in which antibody production and red cell destruction take place. The extent to which the spleen con-

tributes to hemolysis is difficult to assess in any given case. Recent experimental observations using red cells labelled with radioactive chromium may find clinical application. Estimation of the accumulation of the isotope in the spleen as compared with other organs may offer an additional criterion for selecting patients for splenectomy.[30]

DIFFERENTIAL DIAGNOSIS

Little difficulty is encountered in distinguishing auto-immune hemolytic disease from most other hemolytic anemias, with the exception of hereditary spherocytosis. Table 15 lists certain critical findings which are most helpful in differential diagnosis.

TABLE 15. *Differentiation of Auto-immune Hemolytic Disease from Hereditary Spherocytosis*

DISTINGUISHING FEATURE	AUTO-IMMUNE HEMOLYTIC DISEASE	HEREDITARY SPHEROCYTOSIS
Inheritance	None	Mendelian dominant
Response to splenectomy	Variable	Clinical cure
Spherocytosis	Present only in crises	Persistent (even after splenectomy)
Osmotic fragility	Normal during quiescence, usually increased during crises	Usually increased (even after splenectomy)
Rate of destruction of normal transfused cells in patient	Rapid in active cases	Normal in most cases
Detectable auto-antibodies	Present in typical cases	Absent in typical cases

Atypical cases of either disease may be encountered. Additional differentiating features may be helpful if a firm diagnosis cannot be readily established.[50]

TREATMENT

Therapy in most cases of childhood auto-immune hemolytic disease is aimed at partial relief of anemia and suppression of hemolysis until spontaneous cure takes place.

Blood transfusion is valuable for temporary relief of anemia and at times may be lifesaving. The benefit is likely to be transient, however, if treatment is limited to transfusion. The greatest values of transfusion are in relieving profound anemia until the effect of ACTH or steroid therapy results in deceleration of the hemolytic process, or in preparing patients for splenectomy. Because the transfused cells may

be rapidly destroyed, jaundice and hemoglobinemia may actually increase in seriously ill patients after transfusion.

Special precautions should be taken in selecting blood for transfusion. Every effort should be made to obtain blood which seems least incompatible with the antibodies in the patient's serum. In the majority of patients the auto-antibodies act as panagglutinins, and all bloods are to some degree incompatible. In exceptional cases it may be possible by careful serologic studies to find donors whose red cells are little affected by the patient's plasma.

In practice a large number of prospective donors may need to be tested in order to select those that appear least incompatible in the indirect antiglobulin reaction.

Some patients with auto-immune hemolytic disease appear to have unusual ability to form antibodies and may become readily immunized by transfusion. For this reason great care must be exercised to detect specific blood group antibodies stimulated by previous transfusion. Since there may be difficulty in distinguishing incompatibility due to dangerous iso-antibodies, transfusions should be given slowly and under close observation. We prefer the use of sedimented red cells rather than whole blood or washed red cells.

The use of ACTH and cortisone and related steroids has been a great advance in the treatment of auto-immune hemolytic disease. Although little is known of their mode of action in this disease, these hormones are at present the agents of choice in treatment of nearly all cases. These hormones are *not* entirely interchangeable,[19] nor are all patients with auto-immune hemolytic disease benefited by them. If a patient fails to respond to high doses of corticosteroids, treatment with ACTH should be tried. In general, we prefer to start therapy with oral prednisone; in desperately ill patients intravenous hydrocortisone or ACTH may be needed during the first day or two of treatment. If a patient fails to respond to conventional doses of adrenal cortical hormones, larger doses must be used (up to 30 mg. per day of prednisone in a one year old infant, or 75 to 100 mg. per day in an adolescent child). Prednisone in small doses may cause fewer side effects than cortisone or hydrocortisone, but large doses long continued produce serious complications.[47] Patients requiring continued large doses of corticosteroids must be carefully observed. Dosage of cortisone or prednisone should be reduced gradually, but only as rapidly as is consistent with continued suppression of hemolysis. Only one patient in our experience has required prolonged continuous therapy with small doses of prednisone.

The results of *splenectomy* in patients with auto-immune hemolytic disease are unpredictable. Although relatively few childhood cases of chronic or recurrent auto-immune hemolytic disease treated by sple-

nectomy have been reported, the results do not seem to be encouraging.[19, 34, 35, 36,47] Accordingly, splenectomy is *not* recommended early in the disease and should be reserved only for those cases which cannot be successfully managed with adequate doses of hormones.* This view of the management of auto-immune hemolytic disease in childhood is similar to that expressed by others relative to adults.[6] Although treatment of auto-immune hemolytic disease remains empirical, the indiscriminate administration of hematinic drugs is condemned.

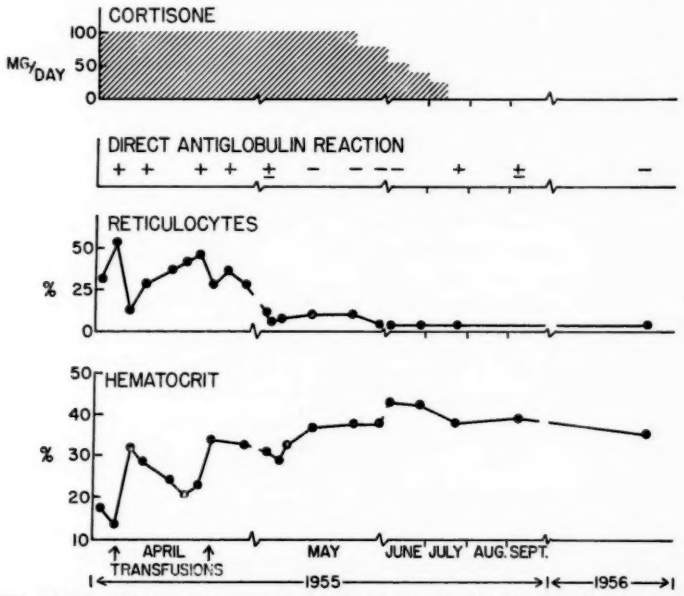

Fig. 11. Idiopathic auto-immune hemolytic disease of relatively short duration showing favorable response to treatment with cortisone. During May, 1955, persistent reticulocytosis provided evidence of compensated hemolytic state in the absence of anemia.

ILLUSTRATIVE CASES

The variable course of the disease, together with the principles of its management, are brought out in reference to specific cases.

CASE I. The 13 month old infant whose course is depicted in Figure 11 is the youngest patient in our series. Minimal symptoms had been present for only 4 days. Pallor

* It has recently been shown that some patients with hereditary spherocytosis (HS) may benefit from treatment with ACTH or cortisone.[7] Therefore the response to hormone therapy may be confusing in attempting to differentiate auto-immune hemolytic disease from hereditary spherocytosis. Splenectomy remains the treatment of choice in hereditary spherocytosis, but not in auto-immune hemolytic disease. Therefore precise diagnosis is important in selection of proper treatment.

was the only sign of anemia, and the spleen became palpable only after a rapid transfusion. Anemia recurred despite prompt initiation of hormone therapy, and persistent fever led to intensive studies which failed to disclose any underlying disorder. During continued treatment with cortisone the evidence of hemolysis gradually diminished. It is noteworthy that although the direct antiglobulin reaction became weaker and then negative as hematologic improvement continued, subsequent recurrence of erythrocyte sensitization was *not* associated with recrudescence of apparent hemolysis. It is not uncommon for the direct antiglobulin test to remain positive for many months without evidence of active hemolysis.

Disease of relatively short duration presents few problems in management. More chronic cases encountered in patients of any age are more difficult to manage.

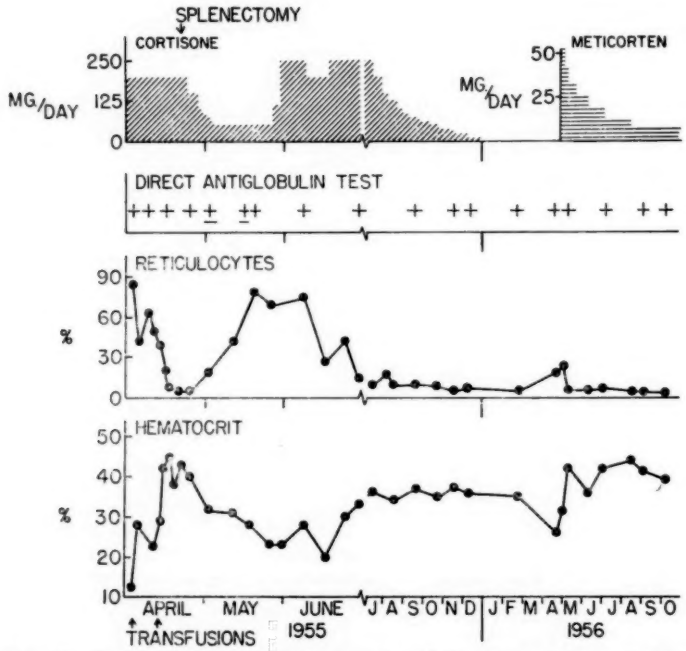

Fig. 12. Chronic idiopathic auto-immune hemolytic disease. Relapse following splenectomy responded favorably to treatment with large doses of cortisone. Recurrence of hemolysis one year after original episode was followed by persistent reticulocytosis and evidence of erythrocyte sensitization.

Case II. Figure 12 shows the course of chronic auto-immune hemolytic disease in an 8 year old boy. Early symptoms of fatigue, mild jaundice, and anorexia led to a presumptive diagnosis of hepatitis. Pallor and splenomegaly ultimately led to hematological investigation. The striking features in this case are the relapse following splenectomy, the chronicity of the process and the tendency to relapse. Throughout 18 months of observation, reticulocytosis and serologic abnormalities have persisted, frequently in the absence of anemia.

In June, 1955, a transient episode of reticulocytopenia was observed during a mild

bout of gastroenteritis. The reticulocyte count fell from 74 to 27 per cent, and the hematocrit dropped abruptly from 28 to 20 per cent. Prolonged or complete reticulocytopenia represents a serious complication in any patient with active hemolytic disease.[3, 5, 20, 37] Although this patient is now in apparent good health, there is no assurance that an underlying disease will not become manifest at any time.

CASE III. The last case cited is remarkable for the relative paucity of symptoms despite severe anemia and prolonged hemoglobinuria. Hemoglobinuria ceased, but little hematological improvement was apparent in spite of continued administration of large doses of prednisone. The development of frank psychosis necessitated withdrawal of hormone therapy. Because the patient tolerates her chronic anemia well, further transfusions have been withheld.

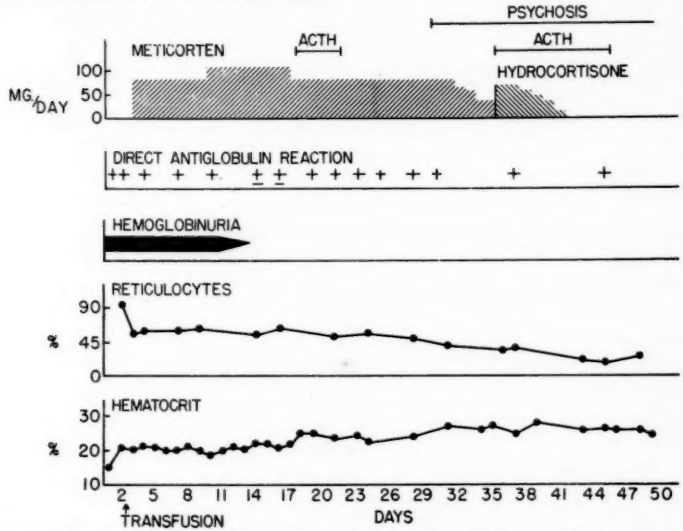

Fig. 13. Auto-immune hemolytic disease in a 14 year old Negro girl. Development of psychosis in this patient required withdrawal of hormone therapy. The spleen has not become palpable during several months of rapid hemolysis. Careful studies have thus far failed to disclose evidence of an underlying disease.

To date we have not considered it advisable to recommend splenectomy or to resume hormone therapy in this patient. Should anemia become more severe, it is possible that treatment with smaller doses of hormones combined with splenectomy might be helpful.

Although we have not personally encountered a fatal case of auto-immune hemolytic disease in childhood, it is likely that occasional patients will succumb from progressively severe disease despite all forms of treatment. As in adults, an underlying disease may manifest itself months or years after the onset of hemolytic anemia.[38]

PAROXYSMAL COLD HEMOGLOBINURIA (PCH)[14, 26]

This is a rare form of auto-immune hemolytic disorder in which the patient produces an auto-antibody which sensitizes erythrocytes on

exposure to cold; upon subsequent warming to body temperature the sensitized erythrocytes are hemolyzed. Serum complement is necessary for red cell sensitization in the cold and for hemolysis at body temperature.

The disease is unique in that it becomes clinically manifest only when the patient is chilled. Intravascular hemolysis results in hemoglobinemia and is followed by hemoglobinuria. Anemia is not a prominent feature of the disease unless repeated bouts of cold-induced hemolysis occur, as during winter months. In some patients the disease is associated with congenital syphilis, but may occur with other disorders.[14]

Diagnosis rests on the demonstration of the hemolysin in the patient's serum, by the so-called Donath-Landsteiner reaction. If the disorder is associated with syphilis, antiluetic treatment results in abatement of the paroxysmal cold hemoglobinuria. In any case the patient should be protected from chilling.

SUMMARY

Auto-immune hemolytic states in childhood are differentiated from other acquired anemias principally by laboratory procedures. The course and response to therapy are unpredictable. Although most cases are idiopathic, the presence of an underlying disease must be carefully sought for in every case, and long-term observation is mandatory. Much progress has been made in understanding the pathogenesis of auto-immune hemolytic states. Continued study of these disorders will undoubtedly lead to improved forms of treatment. Fortunately, most patients respond sooner or later to administration of ACTH or cortisone (or related steroid hormones).

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APLASIA OF ERYTHROPOIESIS

Acute and Chronic Erythroblastopenias or Pure (Red Cell) Aplastic Anaemias in Childhood

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Certainly most doctors are familiar with clinical syndromes caused by cytopenias of the blood. Best known are the acute and chronic forms of thrombocytopenia. Also recognized for a long time is the acute agranulocytosis or neutropenia, though chronic granulocytopenia or neutropenia is less often diagnosed. It had been expected that the analogous forms would occur in erythropoiesis, but their existence has been proved only in the last few years. Ten years ago in a well known textbook of haematology pure red cell anaemia was considered rare enough to be a curiosity and only of theoretical interest. In this paper I will demonstrate that an understanding of the pathogenesis of erythroblastopenias is of great importance today not only in theory, but also in the practical care of haematological problems in children.

HISTORY

What do we understand by the term "aplastic anaemia?" Let me briefly summarize the history of this syndrome.

In 1888 Paul Ehrlich[11] used for the first time the term "aplastic anaemia." Since then it has often been used to describe all kinds of pancytopenias and panmyelopathies, especially in the Anglo-American literature. Likewise the constitutional panmyelopathia of Fanconi, which is a progressive panmyelophthisis with pancytopenia involving not only the erythron, but also the thrombon and neutron, has been called an aplastic hypoplastic anaemia.

In 1922 Kaznelson[20] demonstrated the existence of a true aplastic anaemia without alteration of leukopoiesis and of thrombopoiesis. His

definition of the disease was clear: *progressive normochromic anaemia with no signs of regeneration in the peripheral blood: no polychromasia, no reticulocytes, no erythroblasts; erythropoietic aplasia in the bone marrow with disappearance of all types of erythroblasts; normal leukopoiesis and thrombopoiesis and no enlargement of the spleen or lymph nodes.* He called this disease "pure aplastic anaemia."

In 1927 Baar[3] described under the name "progressive postinfectious erythrophthisis" the occurrence of pure aplastic anaemia in childhood. In 1936 and 1938 Josephs,[19a] Diamond and Blackfan[9] used the term "chronic (congenital) hypoplastic anemia" for a similar disease occurring in infancy and childhood. The progressive anaemia is accompanied by reduction of the reticulocytes in the blood and of the erythroblasts in the marrow, but not by their complete disappearance. In 1948 Owren[23] described an acute "aplastic crisis" superimposed on congenital spherocytosis as cause of sudden severe anaemia. In 1949 the author[13] proved the existence of the same phenomenon occurring in people without a haemolytic constitution due to allergic toxic reactions and called it "acute erythroblastopenia." In 1950 Cathie[6] described under the name of "erythrogenesis imperfecta" chronic forms of aplastic anaemias due to a maturation arrest of the normoblasts. In 1954 Richet, Alagille and Fournier[24] demonstrated the phenomenon of acute erythroblastopenia during anuria. Altman and Miller[2] wrote that patients suffering from chronic congenital aplastic anaemia excrete anthranilic acid in the urine, and finally Bonham-Carter, Cathie and the author[4] presented the first case of an immune type of aplastic anaemia.

In this paper four different clinical-haematological syndromes will be discussed separately: (*a*) acute erythroblastopenia (aplastic crisis), (*b*) chronic erythroblastopenias (pure aplastic and hypoplastic red cell anaemia), (*c*) immune type of erythroblastopenia (immune aplastic anaemia), (*d*) erythroblastopenia (aplastic anaemia) in renal failure.

Pancytopenic states of so-called aplastic and hypoplastic anaemia do not belong to the pure erythroblastopenias and are not included.

ACUTE ERYTHROBLASTOPENIAS (APLASTIC CRISES)

The syndrome of acute erythroblastopenia (Gasser) is defined as an acute and selective damage of erythropoiesis of short duration. As the result of external factors such as toxins, medicaments or infections and of a predisposing constitutional status, a toxic allergic reaction occurs which after about nine days leads to a temporary lack of erythroblasts. In normal erythropoiesis this acute short crisis is not followed by significant anaemia (Case II) and is therefore seldom recognized. Only in constitutional spherocytosis does the acute erythroblastopenia or the

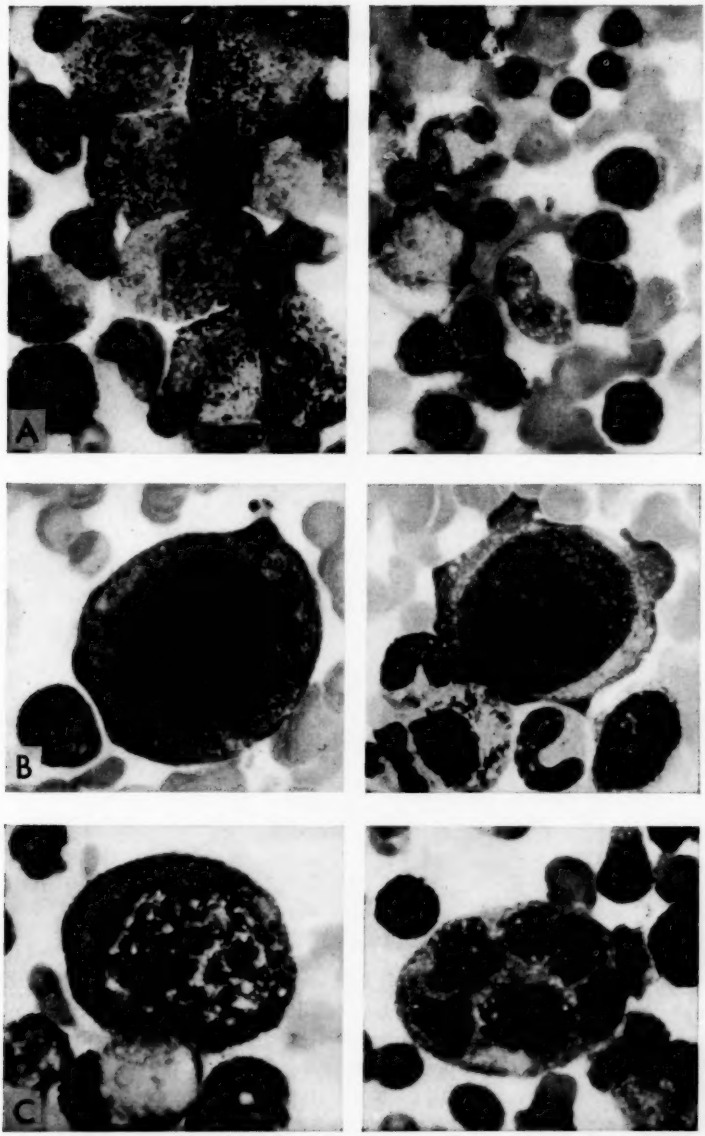

Fig. 14. Bone marrow findings in acute erythroblastopenia. *A*, During crisis there are no erythroblasts in the rich and cellular bone marrow. After crisis a strong erythropoiesis takes place. *B*, Giant proerythroblasts can be found during the aplastic phase of erythropoiesis. *C*, Giant proerythroblasts in the state of polyploid endomitosis.

so-called aplastic crisis (Owren[23]) cause a severe anaemia because of the short life span of the spherocytes (Case I). The existence of an aplastic crisis does not exclude the occurrence of a haemolytic crisis, but the first one seems to be more life-threatening and the main cause of unexpected sudden death in congenital spherocytosis. It is of interest that aplastic crises seldom occur after splenectomy.

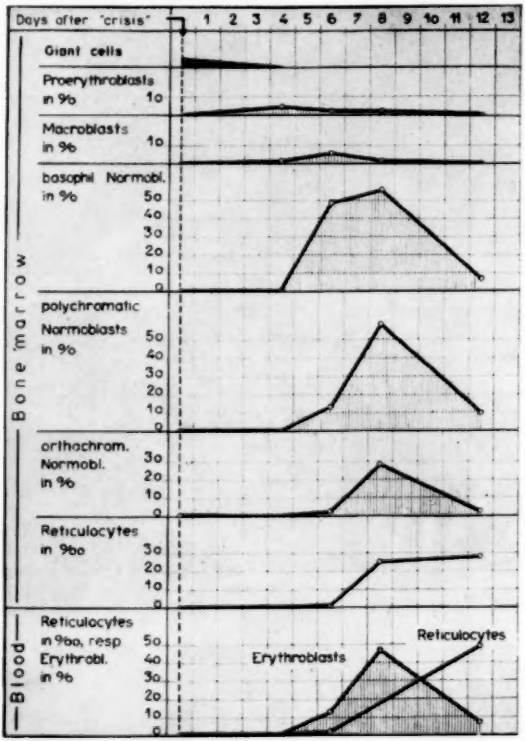

Fig. 15. Maturation study of a single case of acute erythroblastopenia.

Our 20 cases consist of 13 girls and seven boys with ages ranging from six weeks to 13 years. Only three children suffered from congenital spherocytosis. The *causative agents* were drugs or toxins such as barbiturates, Oleum chenopodii, Santonin, Calomel, Pyramidon, insect bites, infection by a virus such as atypical pneumonia and mumps, bacterial sepsis as meningococcaemia, staphylococcaemia, surgical interventions as tonsillotomy and splenectomy. *Family and past histories* reveal *allergic manifestations* such as eczema, asthmatic bronchitis, pseudocroup, anaphylactoid purpura and hypersensitivity reactions to

drugs. On the other hand, these patients also showed an *instability of the blood apparatus* which we called "dyshaemia," such as a tendency to eosinophilia, haemolytic anaemia, megaloblastic anaemia, and erythroblastic reactions. Thus acute erythroblastopenia occurs also in patients suffering from other cytopenias as chronic erythroblastopenia, chronic thrombocytopenic purpura and chronic granulocytopenia. To be men-

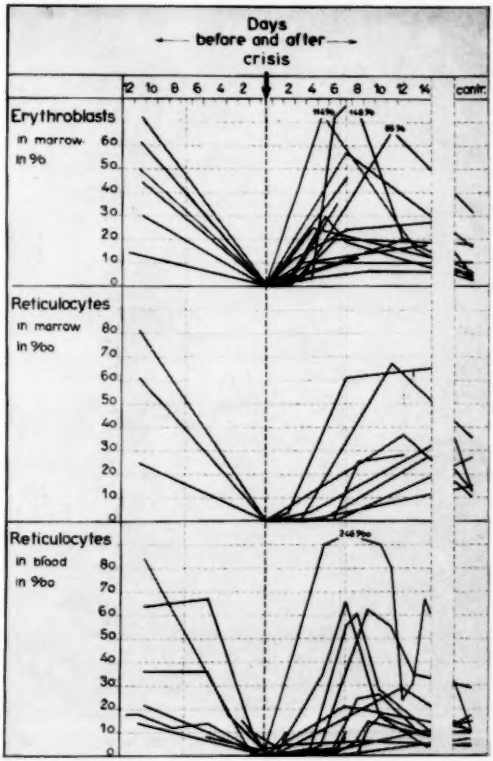

Fig. 16. Summary curves of 20 cases of acute erythroblastopenia. Note the increased number of erythroblasts and reticulocytes after the crisis, which lasts only a few days.

tioned also is the recently described acute form of erythroblastopenia in *anuria*, which will be discussed later.

The *peripheral blood* is, as in all forms of erythroblastopenia, characterized by the absence of erythropoietic activity, i.e., no reticulocytes, no polychromasia. The *bone marrow* is rich in cells with normal neutropoiesis and thrombopoiesis, but lacking the erythroblasts of all maturation states (Fig. 14, A).

A peculiarity is the *occurrence of a few giant proerythroblasts* reminding one of primitive reticular elements (Fig. 14, *B*). Their size is 20 to 60 microns in diameter; their shape varies from round-oval to star-shaped. The generally deep blue protoplasm appears to be easily damaged and is sometimes vacuolated. The nucleus is extremely big, round-oval and of fine, spongy structure. The nucleoli are often big,

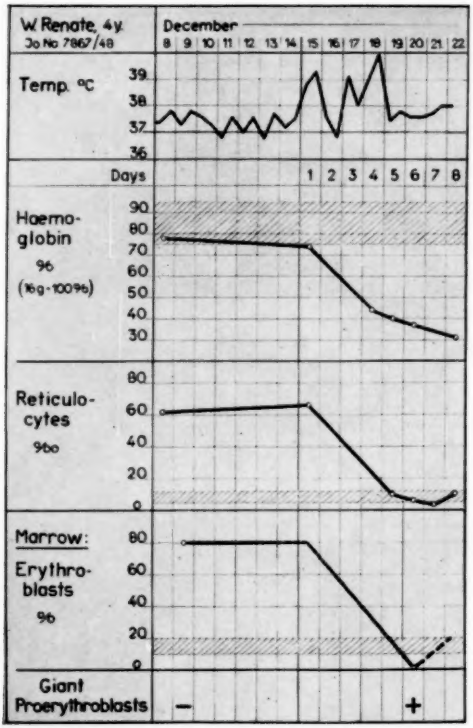

Fig. 17. Case I. Acute erythroblastopenia in congenital spherocytosis (aplastic crisis of Owren). Note the drop of haemoglobin due to the lack of red cell production and short life span of the spherocytes (about 2 weeks).

dark and irregular. Possibly they are remnants from atypical polyploid endomitosis (Undritz[31]), of which Figure 14, *C*, gives examples. Mature normoblasts are seldom seen. According to Weicker,[32] the appearance of giant proerythroblasts is due to the lack of the division of proerythroblasts. They are the potential reservoir for erythroblasts, having the ability to divide eight times and form 256 normoblasts. Eight days were postulated for this to occur, a time which we found empirically in a maturation study of the red cell elements in a single case (Fig. 15).

During recovery all types of erythroblasts are produced in excessive amount, and their number becomes normal only one week later.

The *summary curves* (Fig. 16) of our 20 cases of acute erythroblastopenia show the synchronized appearance of the hiatus as seen in erythroblasts and reticulocytes of bone marrow and blood respectively.

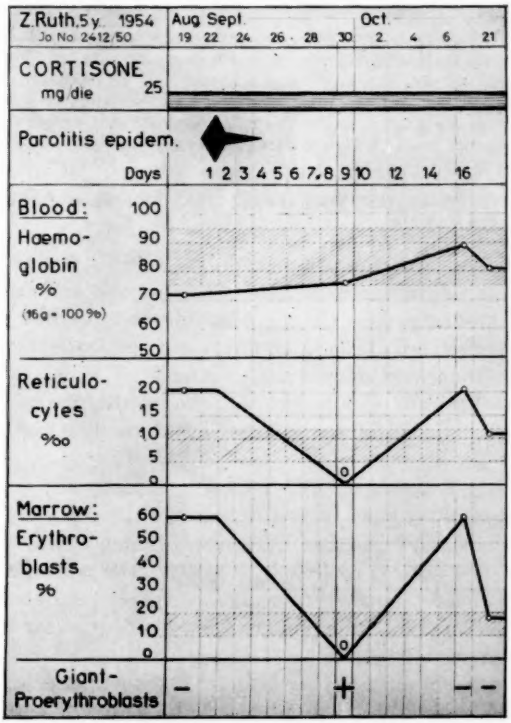

Fig. 18. Case II. Acute erythroblastopenia occurring after mumps in a case of chronic erythroblastopenia, the latter successfully treated with cortisone. There is no drop in the haemoglobin, despite the lack of red cell production, because of the long life span of normal erythrocytes.

A week after the crisis, erythroblastosis in the bone marrow and reticulocytosis in the blood can be seen.

The difference between acute erythroblastopenia (aplastic crisis) in haemolytic disease and in normal erythropoiesis may be shown in two cases, both already suffering from blood disorders. It may be mentioned that aplastic crises have also been recognized in other haemolytic mechanisms such as in acquired haemolytic anaemia (Seip[27]) and

in haemoglobinopathies (sickle cell disease, Chernoff and Josephson[8]).

CASE I. CONGENITAL SPHEROCYTOSIS. During clinical observation a severe crisis, as described by Owren,[23] occurred with fever and a sudden drop in haemoglobin; there was no reticulocytosis; on the contrary, the reticulocytes disappeared from the blood and the erythroblasts from the marrow. Lack of new-formed erythrocytes during one week must lead to a sudden anaemia in congenital haemolytic disease because the life span of these cells is only about 2 weeks.

CASE II. CHRONIC ERYTHROBLASTOPENIA WITHOUT HAEMOLYTIC CONSTITUTION SUCCESSFULLY TREATED WITH CORTISONE. Eight days after mumps there occurred an acute erythroblastopenia. This acute postinfectious form of erythroblastopenia must have another etiology than the chronic form, since it was not influenced by cortisone; the appearance of the giant proerythroblasts is also unusual in chronic erythroblastopenia. In this particular case we found them only twice in 88 bone marrow aspirations.

CHRONIC ERYTHROBLASTOPENIAS (PURE APLASTIC AND HYPOPLASTIC RED CELL ANAEMIA)

In comparison with the acute erythroblastopenia, a completely different kind of disturbance is responsible for the various idiopathic *chronic* erythroblastopenias. In the American literature they have been known as *pure red cell anaemia, chronic congenital hypoplastic anaemia* and *chronic congenital aregenerative anaemia.*

Figure 19 illustrates a simple *classification* of the different types according to the number of erythroblasts in the marrow and of reticulocytes in the blood.

Pure aplastic anaemia shows a complete absence of reticulocytes in the blood and of all types of erythroblasts in the marrow. This is the type that Kaznelson[20] and Baar[3] described. Better known is the *hypoplastic* type, first noticed by Josephs[19a] (1936), Diamond and Blackfan[9] (1938) and called "chronic (congenital) hypoplastic anemia," according to the findings of a reduction of erythroblasts and reticulocytes, but without their complete absence. We must distinguish between those cases with maturation arrest at different stages, whether it be of the basophilic or the oxyphilic erythroblasts. In both types there are few or no reticulocytes in the peripheral blood. In Lelong's[21] case many but only basophilic erythroblasts were present in the marrow, and in Cathie's[6] case all types of erythroblasts were found. Because of the presence of numerous erythroblasts in such cases we chose to call it *pseudoaplastic anaemia.*

Clinical Picture

Chronic erythroblastopenia or chronic aplastic and hypoplastic anaemia is still a rare but important disease. There are no known hereditary familial factors. Both sexes can be affected. Association with malformations is unusual (Case IV), but the physiognomy is some-

times impressive (Cathie[6]). Many children are premature with retardation in growth, even without corticoid treatment. The onset is sometimes in the first months of life, often during the first years. There is no proof that the disease is congenital. The anaemia develops slowly, going hand in hand with general symptoms such as weakness, anorexia, dyspnoea and tachycardia. There are no signs of pyodermic lesions or a

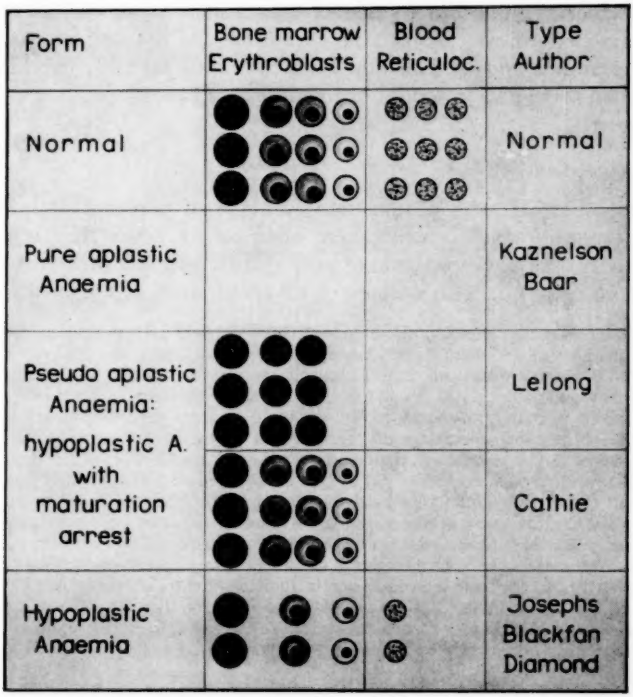

Fig. 19. Classification of the different types of chronic erythroblastopenia (idiopathic chronic aplastic anaemias) reduced to a simple scheme.

bleeding tendency. The spleen and lymph nodes are not enlarged. Exogenous haemochromatosis may develop late in those patients treated with repeated transfusions.

The haemogram shows a normocytic, normochromic anaemia without signs of regeneration: no polychromasia, no reticulocytes. Neutropoiesis and thrombopoiesis are not affected. The bone marrow (Fig. 21, A) is rich with a selective lack of the erythroid series. The serum-iron level is elevated, because the iron is not used for erythropoiesis. Altman and Miller[2] believe this disease is related to a disturbance of

trypotophan metabolism with a pathological elimination of anthranilic acid in the urine. Their findings could not be substantiated by Ruhenstroth* in our cases.

Prognosis

The prognosis of this disease was poor until a few years ago. Only a few patients showed spontaneous recovery after a few years of transfusion therapy. The others died of transfusion haemosiderosis or of shock after transfusions. Since the *introduction of the corticoid therapy* by the author and by Hill and Hunter,[18] both in 1951, the life expectation is much better, most of the untreated cases responding well to cortisone.

Treatment

The following cases demonstrate examples of pure aplastic and of pseudoaplastic anaemia which responded to cortisone; then in comparison, two far advanced cases of pure aplastic anaemia with exogenous haemochromatosis, which were refractory to steroids, are mentioned.

CASE III (identical with Case II; Fig. 20). This 7 year old girl was a premature baby of normal physical and mental development. At the age of 10 months she had measles, followed by weakness and pallor. At 14 months she was admitted to the hospital with a normochromic anaemia of 4 gm. per 100 ml. Over the months reticulocytes disappeared completely from the blood and erythroblasts from the bone marrow. Treatment with the usual haematopoietics failed, and transfusions gave only temporary symptomatic improvement. Seven months after the recognized onset of her disease we started treatment with cortisone and had an unexpected success. On high dosage of cortisone the marrow produced a great number of erythroblasts, followed by reticulocytosis and improvement of the anaemia. With relatively small maintenance doses the bone marrow continued to form erythroblasts satisfactorily, and no further transfusions were necessary. When cortisone treatment was stopped, erythroblasts again disappeared from the bone marrow. Then further treatment with cortisone was necessary and was again successful. Two years ago we tried for the fifth time to stop the treatment, and this time erythropoiesis continued; we really hope the child is cured, because she is still perfectly well without therapy.

I should like to emphasize further that the girl has no haemosiderosis and also no disturbance of the endocrine system, after having received 33 gm. of cortisone from November, 1950, till May, 1955.

The *changes in the bone marrow* are seen in Figure 21; at the top the bone marrow is rich and ripe, but without erythroblasts; under cortisone therapy the appearance of all kinds of red cell precursors is evident. When cortisone is withdrawn, the erythroblasts disappear again.

This first trial of cortisone therapy in chronic erythroblastopenia stimulated us and other authors to give the same treatment in other cases with subsequent success.

The next case is an extremely unusual case of hypoplastic anaemia in

* I wish to thank Dr. Ruhenstroth, Max Planck Institute, Tübingen, Germany, for these determinations.

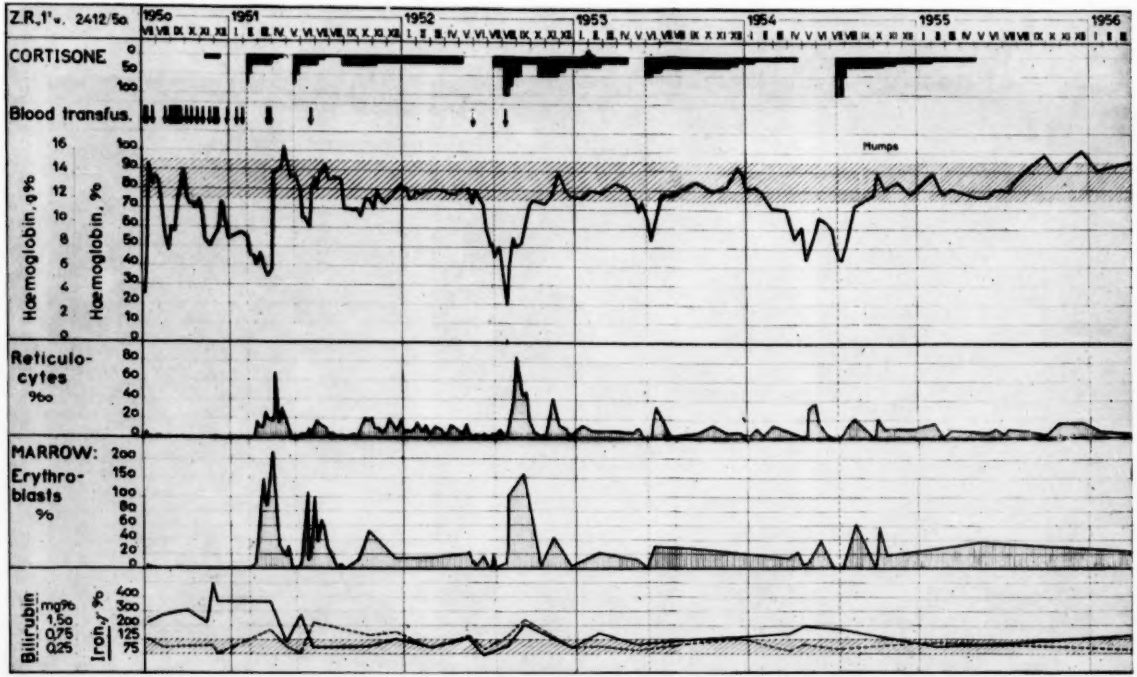

Fig. 20. Follow-up study of Case III (idiopathic chronic erythroblastopenia, identical with Case II) treated since 1950 with cortisone. Thrombocytes and leukocytes were always normal.

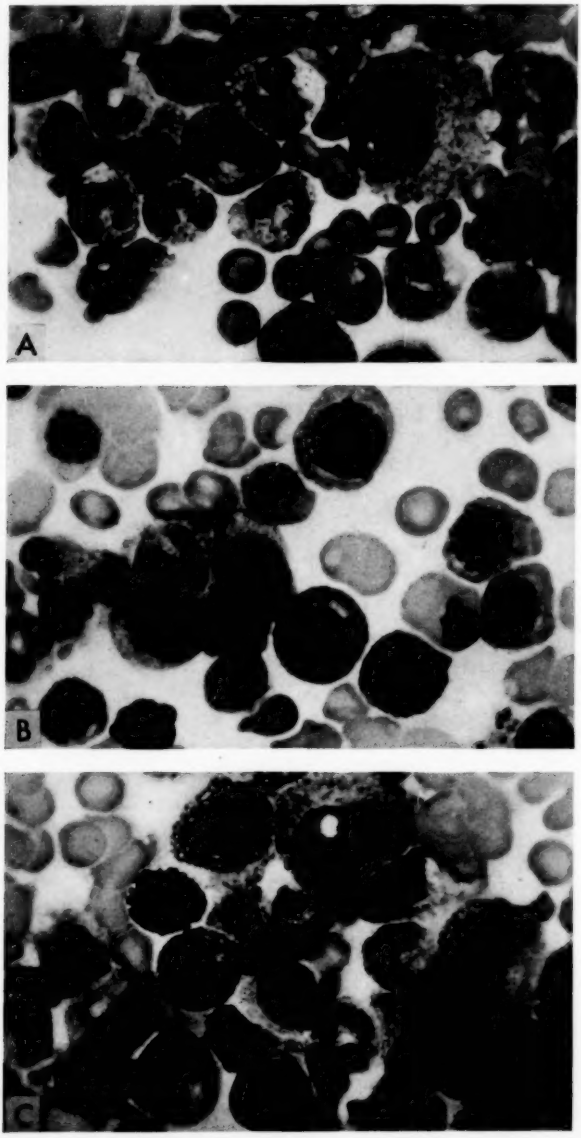

Fig. 21. Changes in the bone marrow in chronic erythroblastopenia (pure aplastic anaemia) under cortisone treatment. *A*, Before treatment no erythroblasts were seen in the rich bone marrow. *B*, Under cortisone therapy there appeared all kinds of red cell precursors. *C*, After withdrawing cortisone the erythroblasts disappear again.

a child with multiple congenital anomalies, not to be mistaken for the Fanconi type.

CASE IV. The 2 year old girl suffers from many malformations such as atrial septal defect, cleft palate and mental and physical retardation. Since birth she had signs of a refractory anaemia, requiring blood transfusions. She entered the hospital at the age of 4 months for surgical treatment and was then transferred to the medical service for analysis of her anaemia. The child had a normochromic, normocytic anaemia of 8.5 gm. per 100 ml., with 2.4 per cent reticulocytes and a normal osmotic fragility test. The marrow contained 13 per cent erythroblasts. Serum iron and bilirubin were normal. Coombs test was negative. The child needed blood transfusions despite her cellular bone marrow with a normal or even increased number of erythroblasts and a satisfactory number of reticulocytes in the blood. Later intensive therapy with folic acid, iron and hydrochloric acid failed to prevent the fall of haemoblobin, which fell to 5.3 gm. per 100 ml. The blood picture was normocytic with 0.3 per cent reticulocytes and 20 per cent erythroblasts without maturation arrest present in the rich bone marrow preparation. At that time we started 30 mg. of prednisone daily and were much surprised to find 9 days later a rise in the haemoglobin due to a reticulocytosis of 10 per cent. From that time we treated only with prednisone. In the last few months we had to increase the dose with renewed success, the haemoglobin rising again to a normal level of 12.8 gm.

In this case we have no proof of an abnormal haemolytic mechanism. It is difficult to explain the kind of anaemia and the benefit derived from cortisone treatment. We think this patient belongs to the pseudo-aplastic type, having normoblasts in all stages of maturation. A refractory anaemia of this type should therefore be submitted to a trial with cortisone even if there is no certain proof of erythropoietic hypoplasia.

The good results of cortisone treatment in fresh cases of chronic erythroblastopenia of the pure aplastic type stimulated us to try it also in patients with the same disease who had been kept alive with transfusions for many years and already showed signs of severe haemosiderosis or exogenous haemochromatosis. Unfortunately cortisone therapy failed in two of these children (Ablin and Gasser[1]).

We may *conclude that cases with haemosiderosis* will end fatally with or without cortisone treatment, the haemosiderosis leading to non-response of the erythropoietic tissue. Therefore we emphasize that cortisone treatment in chronic erythroblastopenia should be given as early as possible before the development of haemosiderosis, especially since there is no treatment of the haemosiderosis itself. *Iron therapy is strongly contraindicated.*

Corticoids are today the treatment of choice. Transfusions should be used sparingly. A few good results are known following treatment with cobalt (Seaman[26]) as well as after surgical interventions such as splenectomy and thymomectomy (Humphreys and Southworth[19]). I would suggest that in cases of local lymphadenopathy lymphadenectomy should be attempted. Marshall Chalmers[7] demonstrated the effect of combined therapy including thymomectomy, splenectomy and ACTH. Of course, all essential dietary requirements for red cell formation must

be supplied. In the evaluation of any form of therapy it must be considered that spontaneous recovery is possible too.

In an attempt to clarify the effects of these therapeutic measures and to discuss at the same time the etiological aspects of chronic erythroblastopenia I would like to offer the following hypothesis as depicted in Figure 23.

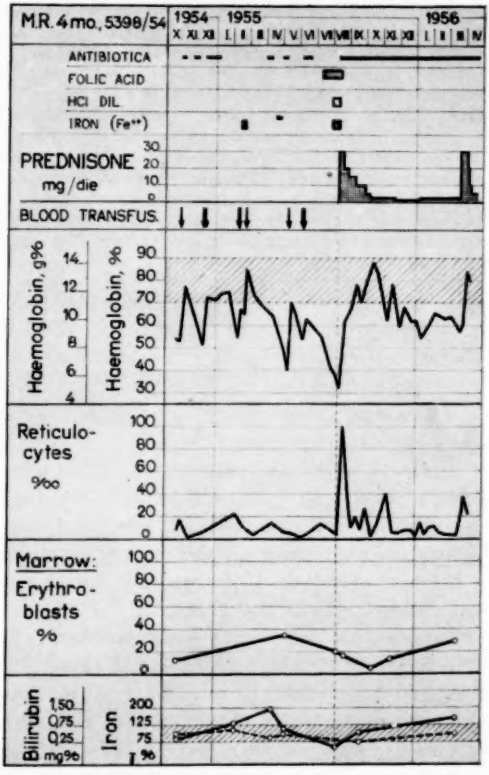

Fig. 22. Case IV. Hypoplastic form or pseudoaplastic type of chronic erythroblastopenia. Cortisone therapy effective in a seemingly not aplastic anaemia.

We may suppose a *balance* between the neuro-endocrinologic system on one side and the lymphatic system on the other. The neuro-endocrinologic system, that is, the hypothalamic-pituitary-adrenal axis, is responsible for stimulation of erythropoiesis and neutropoiesis as well as for inhibition of lymphopoiesis. Contrariwise, the lymphatic system, which includes the lymph glands, thymus and spleen, may be postulated to stimulate lymphopoiesis and inhibit erythropoiesis and neutro-

poiesis. Evidence to substantiate this theory may be found in the therapeutic effect of ACTH and cortisone, which cause a lymphopenia due to their inhibitory effects on the lymphatic system and, directly or indirectly, to further erythropoiesis and neutropoiesis. Hyperactivity of the entire lymphatic system, or single organs thereof, such as a thymoma, lymphatic hyperplasia or hypersplenism, stimulates lymphopoiesis and reduces erythropoiesis and neutropoiesis. Substantiating this relationship are those patients suffering from chronic neutropenia with

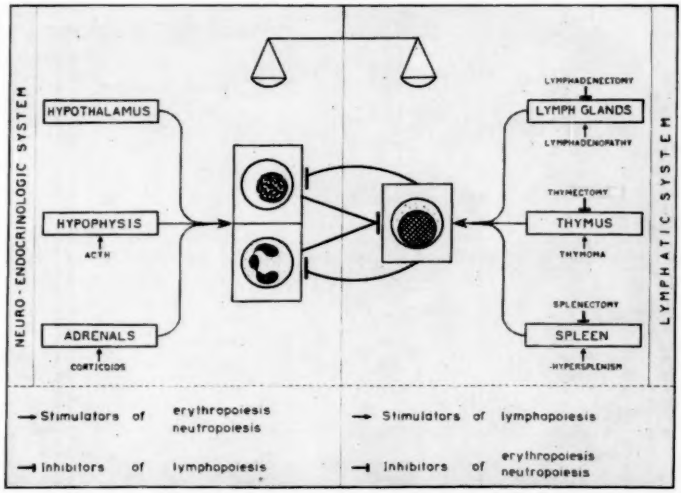

Fig. 23. Scheme of hypothetic regulation of erythropoiesis, neutropoiesis and lymphopoiesis and of therapeutic possibilities.

lymphocytosis and erythroblastopenia. Thus the beneficial effect of splenectomy and thymomectomy, which are known, and of lymphadenectomy, which we postulate, may be anticipated.

IMMUNE TYPE OF ERYTHROBLASTOPENIA (IMMUNE APLASTIC ANAEMIA)

For some time speculations about the relationship between erythroblastic aplasia and antigen-antibody reactions have been made (Smith,[29, 30] Loeb, Moore and Dubach,[22] Rohr,[25] Gasser).

Figure 24 shows a simplified interpretation of the pathogenesis involved in aplastic anaemias which are here considered in the light of their immune etiology. This concerns the occurrence of aplastic anaemia or pseudoaplastic anaemia in haemolytic anaemias. In the usual haemolytic anaemia the mature erythrocytes are destroyed and the reticulocytes in the blood as well as the erythroblasts in the bone mar-

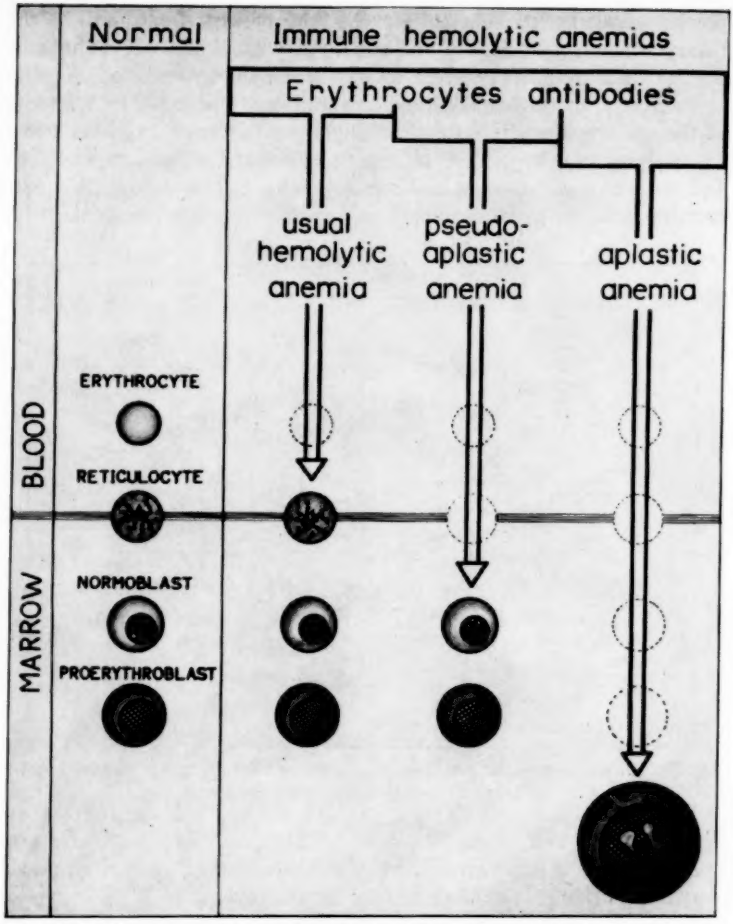

Fig. 24. Scheme to explain our hypothesis of the pathogenesis in immune haemolytic anaemias. Erythrocytic antibodies occasionally destroy not only normal erythrocytes, but also reticulocytes or even erythroblasts in the bone marrow, producing pseudoaplastic and aplastic forms of immune haemolytic anaemias.

row are in excess. On rare occasions the immune antibodies destroy all non-nucleated red cells in the blood, including the reticulocytes; meanwhile the bone marrow still shows a hyperplastic erythropoiesis. We called this type *immune pseudoaplastic anaemia* because of the resemblance to the chronic pseudoaplastic anaemia due to maturation arrest at the late normoblast level (Fig. 19). If the antierythrocytic substance not only works on the erythrocytes and reticulocytes, but attacks

also the erythroblasts in the bone marrow, we have the picture of an *immune pure aplastic anaemia* resembling the type of Kaznelson and Baar due to erythrocytic immune antibodies. Whether they are blood group-specific antibodies or blood group-nonspecific auto-antibodies, antierythrocytic substances may cause these various types of aplastic anaemia. The following are only two of many examples of aplastic anaemia caused by these two different types of erythrocyte antibodies.

Case V (Fig. 25). This 6 week old girl with Rh-incompatibility had received an exchange transfusion of 800 ml. of Rh-negative blood at another hospital. No reticulo-

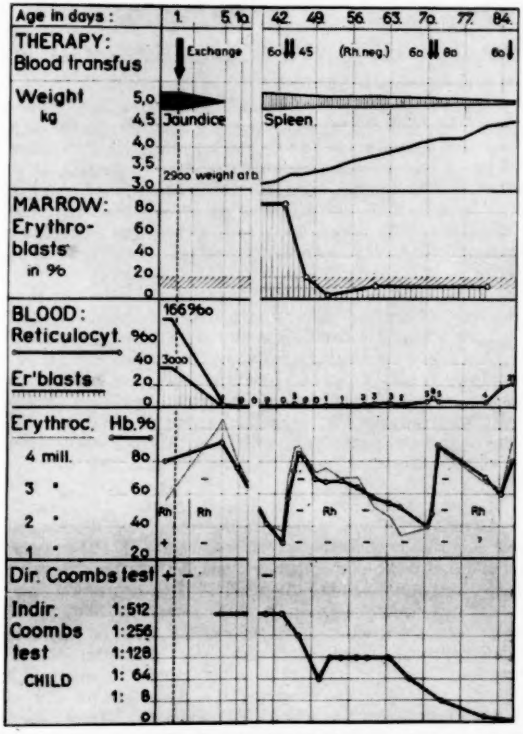

Fig. 25. Case V. Follow-up study of a pseudoaplastic anaemia caused by blood group-specific immune antibodies of the Rh type.

cytes were to be seen 10 days later. However, not much attention was paid to this, since the child showed no anaemia and the direct Coombs test was negative. In the second week of life the baby became steadily paler but not jaundiced, and her spleen was large and hard. She had a normochromic anaemia of 4.8 gm. per 100 ml., and in the blood no reticulocytes were found. In contrast, in the bone marrow there was an increased number of erythroblasts without maturation arrest. The erythrocytes in the child's blood were still Rh-negative, as after the exchange transfusion. The con-

trast between the bone marrow and the peripheral blood could be explained only by supposing a destruction of normocytes as well as of reticulocytes. Indeed, we found a high titre of incomplete Rh-antibodies still present in the child's serum, while the *direct* Coombs test was negative because there were no Rh-positive erythrocytes in the peripheral blood. Only after several weeks of observation, i.e., in the third month, did the indirect Coombs test decrease and reticulocytes appear.

Here there is a case, then, *of pseudoaplastic anaemia caused by blood group-specific antibodies*. Similar but less severe cases have been observed in our hospital. In 1956 Giblett, Varela and Finch[17] discussed another case of Rh-incompatibility in a newborn, accepting our hypothesis that antibodies may act even against erythrocyte precursors. This hypothesis was suggested by observation on the next case, one of acquired haemolytic anaemia due to nonspecific auto-antibodies which evolved into one of aplastic anaemia.*

CASE VI (Fig. 26). This 9 year old boy was a premature suffering since his first years from nutritional disturbances and upper respiratory tract infections. He has an aunt who had had a splenectomy performed because of essential thrombocytopenia. Both our patient and his brother suffered from eczema. Since smallpox vaccination at the age of 2 he showed repeated generalized lymph node swellings with a predilection for the left axillary nodes. The blood picture was normal, and the biopsy of a lymph node was diagnosed as simple hyperplasia. There were no signs of reticulosis or leukemia.

In April, 1952, at the age of 4½, he became steadily paler. At the beginning of June, 1952, he was not only pale, but also had lymphadenopathy and an enlarged spleen. He had an acquired haemolytic anaemia with marked peripheral reticulocytosis and erythroblastosis in the bone marrow. The serum bilirubin was normal, but the urobilin output in the stools was quantitatively increased. Serologically were found incomplete nonspecific auto-antibodies with a positive direct and indirect Coombs test. Before treatment was begun the haemoglobin fell to 4 gm. per 100 ml. Initially, intensive therapy with cortisone and with transfusions brought about a normalization of the haemogram and a negative Coombs test. For almost one year this acquired haemolytic anaemia was clinically latent. Gradually, however, the Coombs test became positive once more, and in August, 1953, the boy presented again a severe anaemia of 2.7 gm. with signs of strong auto-antibody activity. For the first time no reticulocytes were present in the blood and only about 1 per cent erythroblasts in the bone marrow. Since cortisone alone did not bring about any improvement, splenectomy was performed in September, 1953, after preparation with cortisone, ACTH and blood transfusions.

Postoperatively, occasional reticulocytes were found, and the negative Coombs test showed the disappearance of auto-antibodies. After discontinuance of the ACTH treatment there again appeared signs of acquired haemolytic anaemia in an aplastic phase with *disappearance of reticulocytes and of erythroblasts* for a period of months. The destruction of erythrocytes was abnormally accelerated, and the anaemia could be held in check only by intensive blood transfusion therapy.

At the beginning of 1954 massive cortisone treatment of 250 mg. daily was given. Nineteen days later the bone marrow had completely changed. For the first time in several months 68 per cent erythroblasts in all degrees of maturation were found in the bone marrow and 7.5 per cent reticulocytes in the blood. The haemoglobin level increased without further transfusions. During the last 2½ years he has continued to do well on high doses of cortisone and later prednisolone. This has been

* I wish to express my thanks to Dr. Bonham-Carter and Dr. Cathie of Great Ormond Street Hospital, London, for having offered me some of the haematological and clinical data and the possibility to investigate this case jointly.

necessary to prevent the appearance of auto-antibodies despite the inconvenience of an iatrogenic Cushing's syndrome.

From the presentation of these two cases and those recently reported by Eisenmann and Dameshek,[12] Seip,[27] Bousser et al.,[5] and Giblett, Varela and Finch[17] we believe that antibodies can damage not only peripheral blood, but also bone marrow cells.

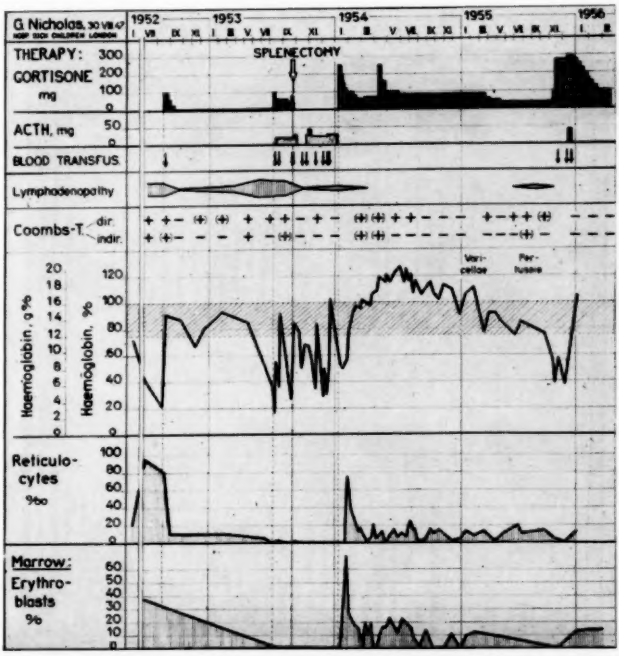

Fig. 26. Case VI. Follow-up study of a pure aplastic anaemia caused by blood group-nonspecific auto-antibodies in the course of an acquired haemolytic anaemia. Life-saving therapy with corticoids is shown.

Other similar disease pictures have been observed without clear evidence of proved antigen-antibody mechanisms. However, we believe that the antierythrocytic substances are present in a form not detectable by our present methods.

ERYTHROBLASTOPENIA (APLASTIC ANAEMIA) IN RENAL FAILURE

Of special interest are the *relations between the erythropoietic system and renal failure.* For many years studies have been made about the so-called hypoplastic anaemia in renal failure, and just in the last

few years the French authors Richet, Alagille and Fournier[24] were able to demonstrate the occurrence of acute *erythroblastopenia in anuria*. Erythroblastopenia occurred mostly on the fifth day of anuria and lasted longer than in those due to an allergic etiology which we have observed (see p. 446). We have studied 30 cases of uraemia and discovered that there is no strict relation between the degree of azotemia and the percentage of erythroblasts in the bone marrow (Fig. 27). Both erythroblastopenia and erythroblastosis in the marrow were associated

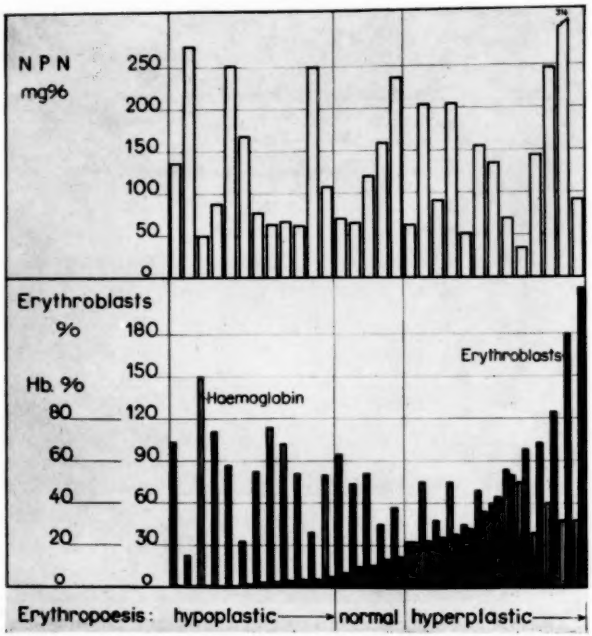

Fig. 27. Relationship between nonprotein nitrogen level, haemoglobin values and number of bone marrow erythroblasts in 30 cases of uraemia.

with all degrees of azotemia. There is, however, some relationship between the degree of anaemia and erythropoiesis. Children with erythroblastopenia are generally less anaemic than those with erythroblastosis, probably because of the haemolytic mechanism involved in the latter state. Five children revealed complete lack of the erythroblasts, i.e., values below 1 per cent. Four were patients with chronic renal failure, three of them suffering from congenital malformations such as renal agenesis, heterotopia of the kidney, megaloureter, aplasia of the bladder muscles or polycystic kidneys. The fourth child died of small contracted kidneys resulting from a rare familial disease, oxalosis.

These four cases would fit well into the known group with depression of erythropoiesis due to nonelimination of toxic antierythrocytic substances. Only one of these erythroblastopenias occurred during *acute* renal failure with complete anuria. He suffered from so-called *acute haemolytic-uraemic syndrome* (Gasser, Gautier, Steck, Siebenmann and Oechslin[16]).

Case VII (Fig. 28). This 14 month old boy was admitted with acute purulent pleuropneumonia. One day after penicillin therapy was started he became steadily

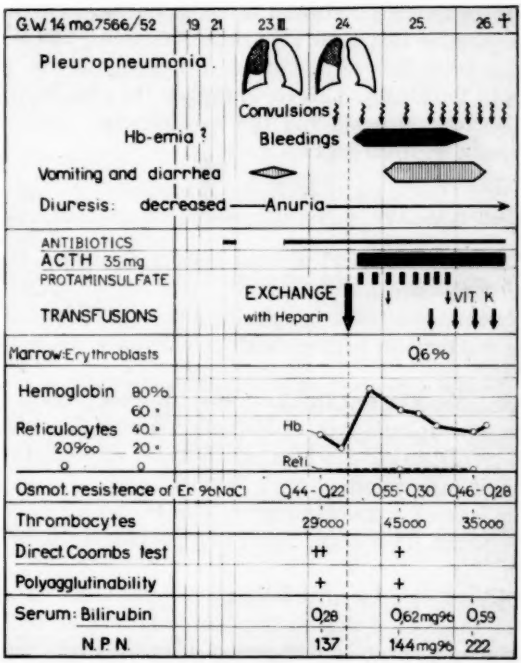

Fig. 28. Case VII. Acute erythroblastopenia in a case of haemolytic-uraemic syndrome.

paler, lost consciousness and had tonic-clonic convulsions. The haemoglobin dropped to 2.6 gm. per 100 ml. The presence of incomplete auto-antibodies was demonstrated by a strongly positive Coombs test and the phenomenon of polyagglutinability. In spite of the severe anaemia the marrow erythroblasts were only 0.6 per cent and the reticulocytes in the peripheral blood 0.1 per cent. A marked thrombocytopenia was also present, and a severe haemorrhagic disorder developed. In spite of ACTH, exsanguino-transfusions and simple transfusions the nonprotein nitrogen continued to rise and the anuria was not influenced. The boy died on the fourth hospital day, and autopsy revealed in addition to a pulmonary abscess secondary to a pulmonary infarct, typical bilateral renal cortical necrosis as described by Sheehan and Moore,[28] but no microscopic evidence of thrombotic thrombocytopenic purpura (Moschkowicz-Symmer-Singer syndrome).

This patient showed all four cardinal symptoms of the haemolytic-uraemic syndrome: haemolytic anaemia, acute renal failure, haemorrhagic diathesis and central nervous system dysfunction evidenced by convulsions. Also he demonstrated the seemingly paradoxical situation of a failure of bone marrow response (erythroblastopenia) in the face of an acquired haemolytic process due, no doubt, to antierythrocytic antibodies.

The etiology and pathogenesis of this syndrome are not at all clear. We have thought of allergic mechanisms as sensitization after infections or drugs, or through destroyed tissue, analogous to a Herxheimer reaction. It is possible that some kind of autoimmunization takes place, auto-antibodies being formed against the blood cells as well as against small arteries in the kidney. This could explain the simultaneous occurrence of haemolytic anaemia and the type of renal failure we have observed, namely, bilateral cortical necrosis.

COMMENTS AND SUMMARY

Four clinical haematological pictures characterized by erythroblastopenia or reticulocytopenia are discussed:

1. Acute erythroblastopenia or aplastic crisis of erythropoiesis due to toxic allergic reactions in patients with or without a haemolytic anaemia.

2. Idiopathic chronic erythroblastopenia or pure aplastic red cell anaemia: the pure aplastic type of Kaznelson-Baar and the hypoplastic form of Josephs-Diamond-Blackfan. Both types respond to corticoid therapy in their early stages.

3. Immune type of erythroblastopenia due to specific or nonspecific erythroid antibodies. They occur either in a pseudoaplastic or in a pure aplastic form.

4. Erythroblastopenia in renal failure.

In some of these cases acquired aplastic anaemia or erythroblastopenia can arise as a result of destruction of erythrocytes, reticulocytes and erythroblasts in the bone marrow by antierythrocytic substances. There is evidence that there is no complete barrier between the peripheral blood and the bone marrow for the influence of humoral substances affecting erythroid cells. We believe that in many of these cases there is a definite immune basis for erythroblastopenia because of the following reasons:

(*a*) Simultaneous appearance of acute erythroblastopenia and allergic manifestations.

(*b*) Occurrence of pseudoaplastic anemia, i.e., complete absence of reticulocytes in the blood, but increase of the erythroblasts in the bone

marrow, in cases of haemolytic disease of the newborn due to Rh antibodies.

(*c*) Appearance of pure aplastic anaemia (chronic erythroblastopenia) with disappearance of both reticulocytes and erythroblasts in the bone marrow in cases of acquired haemolytic anaemia as a consequence of auto-antibody activity.

(*d*) The success of prolonged corticoid treatment in cases of idiopathic chronic erythroblastopenia. This points to circulating antierythropoietic substances as cause of the illness, even if they are not detectable by present methods.

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ANEMIAS CAUSED BY INFECTIONS AND TOXINS, IDIOPATHIC APLASTIC ANEMIA AND ANEMIA CAUSED BY RENAL DISEASE

JAMES ALEXANDER WOLFF, M.D.

The greatest single cause of anemia in the pediatric age group is infection. Another large and heterogeneous group of anemias of infancy and childhood comprises cases secondary to exposure to toxins and those associated with various forms of renal disease. Among infectional anemias the majority are dependent for the most part on deficient blood production, the remainder being classified as primarily hemolytic. In general, anemias associated with infection tend to be mild or moderate in all age groups. Severe cases usually occur in infants and young children. Anemias caused by toxins or renal disease, on the other hand, are frequently severe, and the severity of the process is not related to the age of the patient. As with infectional anemia, anemias caused by poisons are usually primarily the result of deficient bone marrow function, although in some instances a frank hemolytic process may be encountered.

ANEMIAS DUE TO INFECTION

The pathogenesis of infectional anemia is not clearly understood. Impaired erythropoiesis rather than poor intestinal absorption of iron and other structural components of erythrocytes is the primary defect, although a somewhat decreased red blood cell survival time plays a contributory role. Certain biochemical changes related to the metabolism of the red blood cell occur in the presence of infection, changes which can be produced experimentally in animals. The serum iron and the

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serum iron-binding capacity are decreased, whereas the serum copper and the free erythrocyte protoporphyrin are elevated. Intravenous iron administration will raise the serum iron only temporarily and fails to relieve the anemia. Iron so introduced is shunted rapidly to the tissues and is not readily available for use by the blood-forming centers. Although red blood cell destruction is only moderately accelerated, the bone marrow is unable to cope with this mildly increased demand. Indeed, the usual evidences of accelerated production, such as reticulocytosis, are lacking.

In certain acute infections encountered in the pediatric age group rapid increase in red blood cell destruction may occur and a primarily hemolytic anemia result. Sepsis in the newborn period may produce anemia with signs of acute blood destruction identical with those accompanying severe isoimmunization to Rh or major blood group factors. We have seen acute hemolysis in an older child accompanying a hemolytic streptococcal infection, and similar cases have been reported associated with bacterial endocarditis and pneumococcal sepsis. Acute hemolytic anemia is thought to be caused occasionally by viral infections, although in childhood proved instances of this association are rare. Parasitic infection, especially malaria, may be the cause of acute hemolytic anemia in infants and children.

Relatively minor infections, especially if prolonged or recurrent, are frequently the cause of anemia in infants and young children. Significant anemia in older children ordinarily results only from significant infection. Unexpected anemia in the later childhood years should therefore always arouse suspicion of an underlying severe chronic infection such as renal infection or tuberculosis.

The *symptoms* produced by this type of anemia are ordinarily those common to chronic anemias as well as those resulting from the underlying infection. Varying degrees of pallor will be found. In younger patients, if the anemia is severe, splenomegaly may occur. Because of its slow development, the anemia per se produces few symptoms other than irritability or fatigue.

Infectional anemia is usually normocytic and lacking in signs of active blood regeneration. Anisocytosis and poikilocytosis are either moderate or entirely absent. Varying degrees of hypochromia and microcytosis may be found, especially in infants in whom iron lack may be superimposed. The reticulocyte count is normal or low.

Because impaired erythropoiesis is a primary factor in its pathogenesis, permanent *correction* of infectional anemia requires eradication of the underlying infection. If this cannot be easily and rapidly accomplished, if the anemia is severe, or if immediate elevation of the hemoglobin level is required because of a need for surgical intervention, resort to blood transfusion is necessary. Only temporary correction of

the anemia by transfusion can be expected if the infection persists. As already mentioned, iron administered either orally or parenterally is therapeutically ineffective. Oral or intramuscular cobalt preparations have been reported to be of value. Because of the undesirable side effects of cobalt administration, especially the development of goiter with or without evidences of hypothyroidism, the use of this agent in infectional anemia should be condemned.

The rare case of infectional anemia which is frankly hemolytic may manifest severe symptoms from the anemia itself. There may be clinical jaundice, prostration or even congestive heart failure. Such patients will require immediate blood transfusion.

ANEMIA CAUSED BY TOXINS

Aplastic Anemia

Toxic substances may produce anemia either by depression of bone marrow function or by shortening the life span of the circulating red blood cell. The former mechanism is by far the more common. Interference with hematopoiesis may be limited to the red cell series alone, but frequently involves all the cellular elements, resulting in an aplastic anemia. In some instances the cause and effect relationship between exposure to a toxic agent and the development of anemia is clear-cut. In other cases this relationship may be only suggestive or entirely lacking. Except for possibly a slight difference in prognosis, which will be discussed later, idiopathic cases of aplastic anemia are entirely similar to those in which toxic exposure has been shown.

TABLE 16. *All Cases of Aplastic Anemia*

	NO. OF CASES	PER CENT
1. Idiopathic	191	57.2
2. Prior exposure to antibacterials only	79	23.6
3. Prior exposure to agents other than antibacterials	30	9.0
4. Prior exposure to antibacterials and other agents	34	10.2

Agents responsible for the production of aplastic anemia may be separated into two groups. The first is composed of substances which regularly produce bone marrow toxicity whenever sufficient amount of exposure has taken place. The other and larger group includes agents which are only rarely toxic to the blood-forming centers. Anemia caused by such agents is apparently unrelated to amount of exposure and must be thought of as the result of idiosyncrasy.

We recently reviewed a large series of cases of aplastic anemia. Of the total of 334 patients, 24.3 per cent were under the age of 15 years. There was equal distribution of cases between the sexes. Table 16 lists

the number of patients in whom a history of possible exposure to a toxic compound could be elicited: 57.2 per cent gave no such history and were classified as idiopathic; 23.6 per cent had been exposed to one or more antibacterials shortly before onset of the disease; 9.0 per cent had had exposure to other types of possibly toxic materials; 10.2 per cent gave a combined exposure history.

Of the agents which regularly produce bone marrow aplasia, x-radiation and benzol have been the principal offenders. Intense exposure to ionizing radiation from explosion of atomic bombs, on the part of those who have survived the initial blast effects, has led to development of severe aplastic anemia. Benzol is widely used as an industrial solvent. In our series 6.9 per cent of cases gave a history of previous significant exposure to benzol. All these were adults. In none of our cases could x-radiation be incriminated as a possible precipitating cause of the anemia. Because of its depressant effect on the bone marrow, benzol was used in the past as a therapeutic agent against leukemia. In recent years other chemicals have been introduced into the chemotherapy of leukemia. These include the folic acid antagonists, 6-mercaptopurine, Myleran, nitrogen mustards, triethylenemelamine and numerous others. Most of these, when given in sufficient dosage, will produce severe bone marrow depression. Except as a complication of the treatment of leukemia, these drugs have played no part in the production of aplastic anemia.

A large number of substances have been reported as being occasionally associated with bone marrow depression. These include both drugs and potentially toxic nontherapeutic compounds. Of the latter, DDT, hair dyes and trinitrotoluene are of importance in adults. Except for DDT these agents have not been a potential hazard in childhood. The list of therapeutic agents which have been incriminated as occasionally responsible for aplastic anemia is long. The most important are the new antibacterial drugs and the arsenicals. Those of somewhat less importance are gold preparations, Atabrine and the anticonvulsant Mesantoin.

The role of antibacterial agents in the pathogenesis of aplastic anemia has been the subject of much discussion since the publication in 1952 of a number of fatal cases in patients previously treated with chloramphenicol. The Food and Drug Association surveyed a large number of case records of blood dyscrasias. Of these, 296 were diagnosed as aplastic anemia. Forty-four of the 296 (14.9 per cent) had received chloramphenicol only prior to becoming ill, while 95 (32.1 per cent) had received chloramphenicol in addition to one or more other drugs. Of the entire series of 539 cases of blood dyscrasias, 15 patients had received one other broad-spectrum drug only, six had received streptomycin only, and 19 had received a broad-spectrum plus one or more nonanti-

bacterial drugs. It was concluded "beyond a reasonable doubt that chloramphenicol in certain susceptible individuals causes blood dyscrasias, including aplastic anemia . . . ." In our own series of 334 patients with aplastic anemia, nine gave a history in which chloramphenicol was the only substance which might be incriminated as a pathogenic agent. As to other antibacterial agents, there were 26 cases in which sulfonamides, 19 in which penicillin, and three in which Terramycin were the

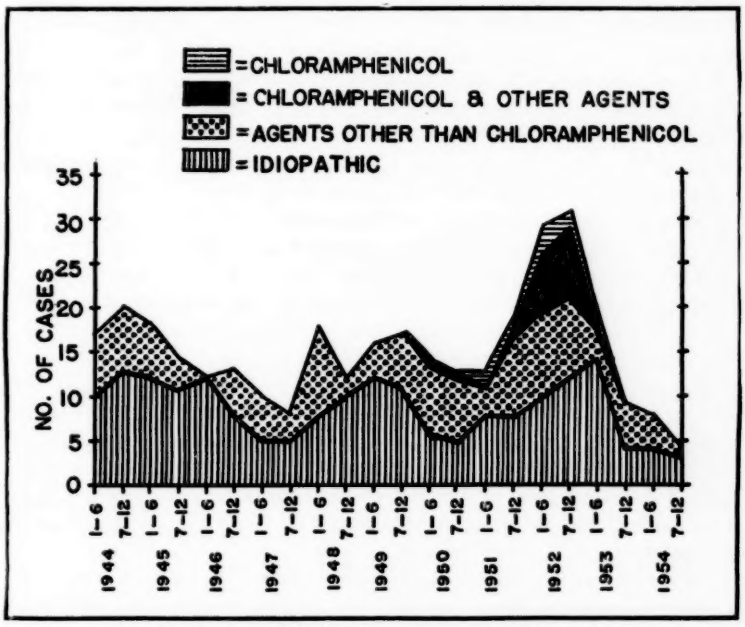

Fig. 29. Semiannual distribution of cases of aplastic anemia.

only possible offending substances. There were no patients in whom Aureomycin or streptomycin had been the only potentially toxic factor. In our series there was a greatly increased incidence of aplastic anemia (Fig. 29) in the year 1952. This increase was due primarily to cases associated with chloramphenicol. As no rise in use of other possible offending antibacterial agents occurred at this time, it is unlikely that more than ordinary interest in aplastic anemia was responsible for the increase. Simultaneously increased interest in chloramphenicol as a potentially toxic compound, however, cannot be excluded as a possible explanation of the peak in 1952. At least from circumstantial evidence, it appears from our analysis of cases of aplastic anemia that chloramphenicol can be causally related to its development. We were unable

to calculate the incidence of aplastic anemia following administration of chloramphenicol. Its occurrence is apparently so infrequent, however, that prohibition of judicious use of this drug is not warranted.

It is of some interest that experimental production of aplastic anemia by chloramphenicol has not been reported, although the same drug has caused anemia in ducks. Smith et al.[29] produced varying degrees of anemia in three dogs receiving 1.0 gm. of chloramphenicol daily. Follette et al.[15] have shown that chloramphenicol, but not chlortetracycline, oxytetracycline, penicillin and streptomycin, interferes with oxygen uptake of leukocytes. Atabrine and urethan also will do this.

Of the organic arsenicals, neoarsphenamine has been reported the most frequently to cause aplastic anemia. Occasionally inorganic arsenic compounds have been incriminated. Mesantoin, gold preparations and Atabrine may also produce aplastic anemia as a sensitivity phenomenon without relationship to duration or amount of dosage.

The possible role of allergy in the production of drug-induced aplastic anemia has been considered by numerous investigators. In our series 21.4 per cent of all types of cases gave a past history of allergy, whereas almost 62.4 per cent in the group exposed to chloramphenicol or other antibacterials had such a history. The incidence of allergic family history was also approximately three times as great in the latter group as in the former. Although these findings may be the result of greater use of antibacterial agents in patients with active allergic manifestations, the role of allergy in drug-induced aplastic anemia has definitely not been disproved. It is also of considerable interest that in slightly over half of the total group of 334 patients no history of preceding infection was elicited. When infection was reported, it was in most instances recurrent rather than chronic.

In our series weakness was the commonest presenting complaint in aplastic anemia, being present in two thirds of the cases. Bleeding was the next most frequent symptom on admission, occurring in half of the patients. It has been our experience that children with aplastic anemia generally appear less acutely ill and have bleeding manifestations more out of proportion to other complaints when they first come for medical care, than do patients with acute leukemia. As the disease progresses, and particularly during its end stages, *symptoms* of more acute toxicity develop. Approximately two thirds of our patients had prolonged periods of significant fever prior to termination of the process. The severity of symptoms, however, is variable. A few patients may have a fulminating onset of severe symptoms with rapid progression to a fatal termination. Another small group runs a fairly chronic benign course with few overt manifestations of the underlying blood disorder. The majority, however, exhibit slowly progressive increase in severity

of symptoms with eventual fatal outcome. Purpura is the commonest physical sign during the course of the illness. Unlike leukemia, in which hepatosplenomegaly is common, enlargement of the liver occurred in only one fourth of our patients, and splenomegaly was even less common. Generalized adenopathy was present in only 2 per cent of the series.

In aplastic anemia there is depression of all the cellular constituents of the peripheral blood. The red blood cells are generally normocytic and normochromic, but may be macrocytic. Reticulocytes are normal or decreased in number. Because of virtual absence of granulocytes, differentiation from leukopenic stages of acute leukemia is difficult from examination of the blood alone. The bone marrow may be hyperplastic, normally cellular, or hypoplastic. When cellular constituents are numerous in the marrow, there frequently appears to be a partial maturation arrest. Differentiation from leukemia by bone marrow aspiration is relatively easy, unless the marrow is hypoplastic. If hypoplasia is present, the majority of cells are small lymphocytes or plasma cells and may be difficult to distinguish from the occasional diluted specimen obtained by marrow puncture in children with acute leukemia. In such instances several aspirations may be carried out at different sites or, preferably, bone marrow biopsy should be performed. It has been our experience that bone marrows which are hyperplastic early in the course of the process usually become hypoplastic as the disease progresses.

The *prognosis* of aplastic anemia is poor. In our series only 3.3 per cent of the patients were considered cured. Sixty-six per cent are known to be dead, the remainder having been lost to follow-up or still under treatment. It is of some interest that only one of the 11 patients who recovered was in the idiopathic group. Of the others, three had been exposed to benzol, two each to Mesantoin, arsenicals and chloramphenicol, and one to sulfadiazine. Although all cases of aplastic anemia have a poor outlook, it would appear that history of exposure to a toxic compound may offer a slightly better prognosis than can be expected in idiopathic cases. Only 7.3 per cent of the patients in our series survived more than five years. More than half were dead in less than one year.

No satisfactory *treatment* for aplastic anemia, either idiopathic or secondary to the administration of known toxic substances, is available at present. Splenectomy has been advocated from time to time, but is generally resorted to as a "last ditch" measure. In our series 35 patients underwent splenectomy. Of these only one recovered. Fifty-one patients were treated with ACTH with no recoveries, and 82 with cortisone, all but one of whom eventually succumbed to the disease. Despite the generally poor prognosis and lack of effective therapy, the occasional

recovery in this disease should be an incentive to leave no stone unturned in the over-all care of children with aplastic anemia. Liberal but judicious use of antibiotics, whole blood or red blood cell transfusions and platelet-rich transfusions may tide the patient over difficult periods and allow an eventual spontaneous recovery.

One more word should be said about the relationship of aplastic anemia and leukemia. In our series 11 patients who had been considered to have aplastic anemia throughout the course of the disease were diagnosed as leukemia either terminally or post mortem. These patients had been followed from one month to as long as two and one-half years before leukemia was diagnosed. In none of these had peripheral blood or bone marrow studies been conclusive until the occurrence of a "blastic crisis" just prior to death.

Acute Hemolytic Anemia

Just as drugs or toxic substances may be responsible for the development of aplastic anemia, so also may they occasionally produce hemolytic anemia. Some regularly produce difficulty when the dose is sufficiently large, whereas others do so only occasionally as a form of hypersensitivity. In the former group naphthalene has been the most frequently encountered offender in our experience.

The occurrence of acute hemolytic anemia as a result of naphthalene toxicity from moth ball ingestion was first reported in this country by Zuelzer and Apt[38] in 1949. They presented both clinical and experimental data implicating naphthalene-containing moth balls. Subsequently it has been shown that of the various breakdown products of naphthalene, alpha and beta naphthols will produce hemolysis experimentally. In the cases reported by Zuelzer, as well as in our own cases, the patient has usually been about two years of age. A history of moth ball ingestion is usually not given unless this possibility is specifically raised by the examiner.

When first seen the child is pale and irritable. There is usually a history of intestinal disturbance, either vomiting or diarrhea, in the previous 24 or 48 hours. Hemoglobinuria may precipitate the request for medical aid. When the child is first seen, anemia is ordinarily severe. The blood smear, as noted by Zuelzer, is characteristic at this stage. Fragmentation of the red blood cells and irregular hemoglobin distribution within the cells are conspicuous. The reticulocyte count and the serum bilirubin are elevated. Hemoglobinemia or hemoglobinuria may occur. In wet preparations stained with methyl violet or brilliant cresyl blue, Heinz bodies can be seen in the red blood cells. Naphthalene derivatives may be identified in the urine for as long as 10 days. The Coombs test is negative.

Treatment consists in the use of blood transfusions to support the patient until all the toxic material has been excreted. Rarely lower nephron nephrosis with oliguria or anuria may occur. In one such instance in our experience complete recovery ensued within 48 hours.

The injudicious use of phenacetin in young infants has also produced several cases of acute hemolytic anemia in our experience. A number of other drugs or toxins if given in sufficient amount may provoke hemolysis. These include acetanilid and lead. Some of the agents which produce hemolytic anemia may also be responsible for methemoglobinemia. Such is the case with benzocaine; we have encountered two recent examples of this sort. The anemia associated with lead poisoning is not clearly understood. Stippling of erythrocytes is a prominent feature. Some increase in destruction of red blood cells occurs, but the anemia is principally dependent on interference with hemoglobin formation.

Some years ago self-limited acute hemolytic anemias of unknown cause, thought to be associated with some type of infection, were classified under the title of *Lederer's anemia*. It is likely that many of these actually resulted from intoxication from naphthalene or other toxic agents or drugs.

A number of substances may produce acute hemolytic anemia on a hypersensitivity basis. These include sulfonamides, especially sulfanilamide, quinine and primaquine. Inhalation or ingestion of fava beans will do the same. In favism the direct and indirect Coombs tests have been reported positive in 80 per cent of cases.

The *symptoms* of acute hemolytic anemia due to drug intoxication are usually acute. There is sudden onset of pallor often accompanied by weakness or irritability. Jaundice is usually not apparent. There may be splenomegaly, especially in the younger patients. As mentioned previously in the discussion of naphthalene poisoning, hemoglobinuria may be the presenting symptom, which may in some cases lead to the development of hemoglobin deposition in the renal tubules, with resulant oliguria or anuria.

Treatment of hemolytic anemia secondary to drug toxicity is primarily that of maintaining the hemoglobin and red blood cell levels until hemolysis has subsided following elimination of the toxic material. This can be done by means of transfusions of either whole blood or packed red blood cells. If oliguria or anuria develops, careful control of fluid and electrolyte administration is essential until adequate renal function is restored. When hemoglobinuria or hemoglobinemia first becomes manifest, alkalinization may be useful prophylactically to prevent deposition of hemoglobin in the renal tubules.

After renal function has been impaired, alkalinization may be hazardous.

ANEMIA DUE TO RENAL DISEASE

Although the severity of anemia is fairly directly proportional to the rise in nonprotein nitrogen in patients with renal disease, the pathogenesis of this type of anemia is incompletely understood. Anemia is regularly found in infants and children with renal insufficiency, regardless of the cause of the nitrogen retention. It is encountered in chronic glomerulonephritis, occasionally in acute glomerulonephritis with elevation of the nonprotein nitrogen, in patients with congenital polycystic kidneys and in chronic pyelonephritis.

The *pathogenesis* of anemia in renal failure is related both to decreased erythropoiesis and to increased blood destruction. The bone marrow shows depression of red blood cell production which is more striking in acute than in chronic renal failure. Little is known about the impairment of erythropoiesis. The levels of plasma iron, copper and free erythrocyte protoporphyrin are normal. The hemolytic nature of the anemia can be demonstrated in some cases by a decreased erythrocyte life span. This may be as short as 16 days or, as in patients with stabilized anemias, it may be normal. In acute renal failure a hemolytic factor may be found which is responsible for causing a shortened survival time of normal red cells transfused into the patient. The anemia is normocytic and normochromic.

The *treatment* of the anemia associated with acute renal failure responds to no known therapy other than blood transfusion. Because the transfused cells may have a shortened survival time, fresh blood should be used when possible. Citrate and plasma protein may bind calcium and aggravate an already existing hypocalcemia; therefore packed red cell transfusions are preferable to whole blood transfusions. In chronic nitrogen retention, anemia may become stabilized at a level which is tolerated by the patient. If stabilization does not occur, periodic transfusions are necessary. The plasma volume may be increased in chronic renal insufficiency. For this reason, and also because these patients are susceptible to nonspecific transfusion reactions, packed red blood cell transfusions should be given again rather than whole blood. The latter is particularly contraindicated if hypertension and congestive heart failure are complications of the renal disease.

Cobaltous chloride has been shown to have a beneficial effect on the anemia by improving erythropoiesis. As mentioned previously, toxic complications of cobalt therapy have been sufficiently common to make use of this agent unwarranted in chronic renal disease. Iron

therapy is ineffective except in those cases in which associated blood loss has occurred.

SUMMARY

The anemia of infection is usually primarily the result of decreased erythropoiesis. Hemolysis may play a secondary role. In certain infections the anemia may be frankly hemolytic. Eradication of the infection is necessary before the anemia can be relieved. Occasionally blood transfusions must be given to bring about prompt temporary correction of anemia.

Anemia produced by toxic agents may result from decreased blood production or from hemolysis. The anemia may be unassociated with depression of other blood elements or may be part of a pancytopenia. Aplastic anemia may also be idiopathic. Aplastic anemia of any type has a poor prognosis; treatment in these cases is unsatisfactory. Hemolytic anemias produced by drugs or toxins have a better outlook. Recovery usually occurs with the aid of blood transfusions after withdrawal of the toxic material.

Anemia produced by renal failure is related both to decreased erythropoiesis and to increased blood destruction. Treatment is unsatisfactory. Prognosis is dependent upon the underlying renal disease.

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IRON DEFICIENCY ANEMIA

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The application of knowledge gained about specific vitamin deficiency diseases since the first part of the century has led to a marked diminution in their incidence. This is indeed fortunate, since death or permanent disability was often the end result. Long before the discovery of vitamins, iron was known to be present in the ash of blood and to be effective in alleviating anemia in some patients, but from time to time the value of iron was discredited. This was indeed unfortunate, since iron deficiency anemia continues to be more prevalent than it should be; the incidence appears to have remained unchanged in this hospital and clinic during the past 20 years.[10] Although iron deficiency anemia per se seldom causes death, it may predispose to serious infections or, with the frequently associated pica, to lead poisoning and thus in turn to a fatal outcome or to persistent neurological sequels. Until iron is made as readily available as certain vitamins, it will continue to be the physician's responsibility to recognize, prevent and treat this all too prevalent deficiency state. It would seem worth while to emphasize in this review those aspects of iron deficiency anemia in infants and children pertinent to this responsibility.

AGE INCIDENCE AND RELATIVE FREQUENCY

The relatively high incidence of anemia in the first two years of life compared with the later years of childhood, and the fact that much of this anemia is responsive to iron therapy are apparent from numerous publications[1, 7, 10, 17, 18] as well as from everyday practice. However, few studies have defined in quantitative terms the age incidence of iron deficiency anemia and its relative frequency as a cause of anemia in children. A survey designed for this purpose was conducted in the Children's Hospital in Cincinnati during the summer of 1954.[9] Anemia was considered present if the hemoglobin was below 10.5 gm. per 100 ml.

after the age of two months. If anemia was present by this criterion, thorough hematological observations coupled with the results of the history and physical examination permitted a classification of the anemia as to etiology. Iron deficiency was considered to exist when the peripheral blood smear revealed microcytosis and hypochromia and when values for the corpuscular indices were reduced.

It is apparent from Table 17 that of the 163 children of all ages found to be anemic, 75, or 46 per cent, were anemic as a result of iron deficiency. Average values for the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were 70 cubic microns, 19 micromicrograms and 27 per cent, respectively. As a cause of anemia, iron deficiency far outranked other hematological disorders, and occurred with outstanding frequency between six months and two years. In this series chronic infection ac-

TABLE 17. *Frequency of Iron Deficiency Anemia in Infants and Children*

AGE IN YEARS	TOTAL PATIENTS	TOTAL ANEMIC	IRON DEFICIENCY ANEMIA		
			NO.	PER CENT OF ANEMIC	PER CENT OF TOTAL
0–1/2	176	41	1	2	0.6
1/2–1	74	19	18	95	24
1–2	140	52	42	81	30
2–3	100	18	9	50	9
3–16	659	33	5	15	0.7
0–16	1149	163	75	46	6.5

counted for only 9 per cent of the anemic patients. This high frequency of iron deficiency anemia is at variance with the published reports of other authors. Diamond,[8] for example, in analyzing the hematological data of 3000 patients found but 25 per cent to be iron deficient, while 61 per cent were anemic "secondary to infection." Similarly Baty[1] lists infection as causing 49 per cent of the anemia found in 514 children, with but 13 per cent resulting from a "deficient diet." It is possible that the more widespread use and greater efficacy of antibiotics explain the difference in incidence of anemia attributable to infection in these three studies. Since each of the studies was done within recent years, a more probable explanation for the discrepancy involves the variable use of the term "anemia of infection," as subsequently discussed.

In this group of 75 patients, as in a larger number of children studied in Philadelphia,[25] the high incidence of this form of anemia among patients aged six months to three years is apparent (Table 18). The vast majority of cases occurred in this age range, with few younger than six months or older than three years.

It is possible to criticize each of the several studies just mentioned

on the grounds that only hospitalized patients were included, and in so doing to imply that the incidence of iron deficiency for a "healthy" infant population is not truly represented.

Such a criticism has been met by investigators[23, 30] in this country and abroad.[13] Their observations were based on knowledge that certain hematological disorders can be classified or characterized biochemically and that the "chemical pattern" of iron depletion states is distinctive. The level of iron in plasma or serum is markedly reduced, and the specific beta-1 globulin known to transport iron and variously referred to as siderophilin, transferrin or iron-binding protein is elevated. The percentage saturation of this protein with iron is consequently reduced below the normal level. To illustrate the differential value of such de-

TABLE 18. *Age Incidence of Iron Deficiency Anmeia*

AGE IN YEARS	IN PHILADELPHIA[25] 1953		IN CINCINNATI[9] 1954	
	NO.	%	NO.	%
0–1/2	4	2	1	1
1/2–1	42	26	18	24
1–2	81	50	42	56
2–3	14	9	9	12
>3	21	13	5	7

terminations, the "chemical pattern" found in the normal subject is compared in Figure 30 with that found in patients with iron deficiency anemia and with the anemia of chronic infection. Such chemical data are more sensitive indices to the development of iron deficiency anemia and to the state of the iron stores in the body than are morphologic characteristics. Thus a decrease in serum iron and an elevation in the iron-binding capacity of serum may antedate a reduction in hemoglobin by several weeks; and, after iron therapy, the hemoglobin and hematocrit may return to normal long before normal values for these chemical characteristics are attained. Using these more sensitive criteria, investigators have shown that the "chemical pattern" pathognomonic of iron depletion states is the usual finding in infants who by currently accepted hematological standards are considered "healthy" and "normal."

Thus it seems clear that deficiency of iron not only is the most common cause of anemia in children, but also that it exists in many as a "subclinical" or marginal deficiency state.

ETIOLOGY

Iron deficiency anemia occurs when the supply of iron fails to meet the demand, i.e., when the iron present in "stores" plus that absorbed

from food is insufficient to satiate the body's needs for hemoglobin and myoglobin iron, for iron-containing enzymes and for the small amount of metal lost via excretory pathways. Often in adults and older children it is possible to ascribe the development of anemia to a single cause, i.e., chronic blood loss. In infants, however, it is seldom possible to do so, since multiple factors may be operating simultaneously.

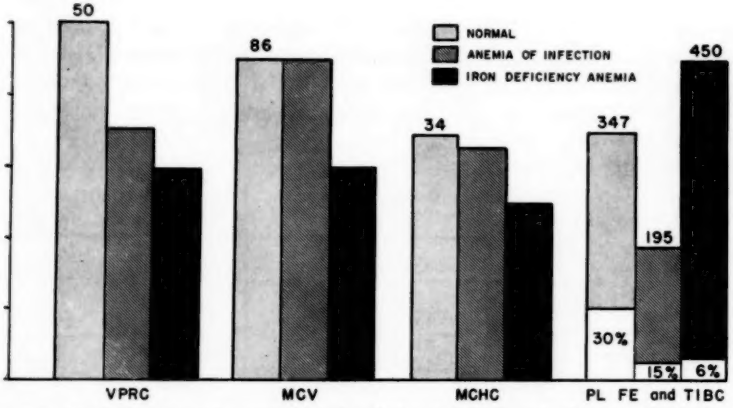

Fig. 30. Comparison of morphological and chemical findings in the normal subject, in anemia of chronic infection and in iron deficiency anemia. Abbreviations: *VPRC*, volume of packed red cells; *MCV*, mean corpuscular volume; *MCHC*, mean corpuscular hemoglobin concentration; *PL FE*, plasma iron; *TIBC*, total iron-binding capacity of plasma.

Role of Enhanced Requirement for Iron

The most obvious explanation for the high incidence of iron deficiency anemia in this age group is the great requirement for iron for incorporation into hemoglobin. Per kilogram of body weight the need is two to three times greater in infancy than at any other period of life.[15] When growing from a 3.5-kg. newborn to a 10-kg. baby of one year, the infant approximately triples his total blood volume and doubles his total body hemoglobin. The requirement is even greater in the prematurely born infant.

In addition to the needs for hemoglobin, there are increased needs for other iron-containing compounds, such as myoglobin and the cytochrome enzymes. Lacking appropriate methods, however, it is not possible, at present, to quantitate the magnitude of these extra-hemoglobin requirements in the human infant. Their importance relative to the incidence of anemia, therefore, remains obscure, but since these compounds seem to have a higher priority for iron than does hemoglobin,

it is conceivable that they might constitute a threat to the hemoglobin iron, at least in some infants.[17, 31]

Since it is common knowledge that two infants of the same age and with similar rates of growth may have markedly different levels of hemoglobin, other explanations than those related to enhanced needs must be sought.

Role of Iron Loss

Because iron, once it has been assimilated in the body, is tenaciously conserved, many investigators have assumed that the problem of iron loss, except as shed blood, can be ignored. Several observations suggest that such an assumption may be fallacious. Some iron is lost with the discharge of cellular elements into body secretions, with the desquamation of skin and mucous membranes, with the growth of hair, and so on, and additional iron may be lost during hemolytic episodes[19] and perhaps during fasting.[15] In the normal adult the daily loss of iron in stool, urine and sweat probably averages about 1.0 mg. per day.[19] Such an amount is negligible to the adult male on an average diet, but may not be to the young woman in whom additional iron is lost via pregnancies and menstruation. There is need for more knowledge of iron loss in the growing infant. The finding that infants under two months of age lose an average of 1.25 mg. of iron per day seems excessive.[28] Nevertheless, until further studies are done, it would not seem rational to exclude completely the small daily loss of iron as a contributing factor in hypoferric anemia in infancy.

Role of Iron Stores

Compared with the adult, who can draw on iron stored in the liver to tide him over a period of critical need, the newborn is probably not so fortified. A recent study[33] indicates that as little as 11.5 mg. of iron may be present in the fetal liver, an amount capable of yielding no more than 3.5 gm. of hemoglobin. The newborn's "store" then resides largely in the hemoglobin compartment. During the next eight to ten weeks of life, with the normal degradation of hemoglobin, iron is released and largely retained for future use. Obviously, then, the infant who begins life with a reduced hemoglobin level and/or a low total blood volume, and therefore with a low total body iron, is immediately at a disadvantage and faces the ensuing months with the necessity of overcoming his handicap by obtaining additional iron from dietary sources. Too often this burden is insurmountable.

Not all factors that govern the newborn's total body iron are known. Some, such as size of the infant, amount of the placental transfusion,

and loss of blood by the newborn, have been well defined. The importance of maternal iron stores is still debated.

The fact that the premature needs more iron than the full term infant is due not only to the more rapid rate of growth, but also to the relatively small blood volume and therefore small "store" of iron at birth. A widely accepted explanation for the premature's greater tendency to have anemia compared with the full term infant is that most of the iron is acquired from the mother during the late weeks of gestation. Recently this concept has been disputed.[33]

It has been shown[34] that early clamping of the umbilical cord increases to a slight but significant degree the severity of anemia in late infancy. This practice deprives the infant of a small blood transfusion equivalent to 30 or 40 mg. of iron. Such an amount, though small, is not inconsiderable. Maximum placental transfusion is accomplished by stripping or delayed clamping of the cord, or both.[6]

It is reasonable to expect that any loss of the infant's blood at the time of birth would be disadvantageous. Such loss may be obvious, e.g., bleeding due to hemorrhagic disease or to faulty ligation of the cord, the replacement of less blood than was withdrawn during exchange transfusion for erythroblastosis fetalis, and so on. On the other hand, significant blood loss may go unrecognized. Thus laceration of placental blood vessels may occur, an infant may bleed into the mother, one twin may bleed into the other.[4, 32]

The observation that the degree of anemia at about one year is greater in infants born to anemic mothers than in those born to nonanemic mothers[29] has been used to emphasize the relation between maternal and infant iron stores. Other observations that anemia is more severe in successive infants of the same iron-deficient mother[10] and that "first born" infants of different mothers are generally less anemic than those "later born"[11] are in agreement with this thesis. In view of this relationship, plus the high incidence of iron deficiency anemia in infancy, one might expect the incidence of iron depletion during pregnancy to be high. Indeed, this occurs so often that one obstetrician[16] has stated, from the standpoint of iron metabolism, that "very few pregnancies qualify as normal." However, since others[37] have failed to find any correlation between anemia in the mother and its subsequent appearance in the infant, this aspect of the problem deserves further study.

Role of Diet

When one considers that the infant begins life with a total body iron content of about 300 mg.[33] and reaches adulthood with 3 to 5 gm., it is apparent that an exogenous source of iron, capable of supplying

about 0.5 mg. per day for 20 years, is necessary. The normal human being absorbs only about 10 per cent of dietary iron,[19] so that a diet providing less than about 5.0 mg. of iron daily or the absorption of subnormal amounts of iron could readily lead to a deficiency of iron. It may, in fact, be necessary for the diet to provide more than this amount, since the needs are greater in the earlier years, since infants up to three months of age may be in negative iron balance[17, 27] since the diet contributes little to the infant's hemoglobin iron during the first three to four months of life.[22] A diet consisting of milk falls far short, supplying as it does only about 1.5 mg. of iron per quart. Furthermore, a well balanced diet contributes not only iron, but ascorbic acid and sulfhydryl groups of protein origin as well, both instrumental in enhancing the assimilation of food iron. If one adds to this accumulated evidence the observation that liver iron stores are scant in late infancy,[24] the critical role played by the diet is obvious.

However, these facts, plus the frequent observation that milk and other foods of low iron content make up the diet of most anemic infants, must not be construed as evidence that all hypoferric anemia results from an inadequate diet or that an adequate diet will prevent all hypoferric anemia. Even a "good" diet may, in the quantities eaten by an infant, provide less than the required amount of iron if the needs are abnormally great or absorption is inefficient. It is not uncommon to find rather severe anemia in infants whose diets are not essentially different from those of nonanemic ones. Brokaw[2] failed to find that the early introduction of cereal, vegetables and eggs had any significant effect on hemoglobin levels during the first year, and Stearns[27] found no difference in iron retention when a diet of milk, egg and spinach was compared with milk alone. By incorporating radio-iron into meat, vegetables and eggs it has been demonstrated[19] that the iron-deficient subject may not absorb iron from these foods any more efficiently than the nondeficient.

The combined evidence, then, suggests that though the physician should continue to recommend a "good" diet, he should not depend solely on it for the prevention and cure of iron deficiency anemia in children. The futility of trying to correct anemia by dietary means alone has recently been stressed again by illustrative calculations in a hypothetical infant.[31]

Role of Infection

Infections may lead to the development of anemia in various ways. Thus accelerated hemolysis, blood loss, suppressed erythropoiesis or various combinations of these may occur. It is well recognized that

anemia in infants is often accompanied by signs of infection. The mere association of anemia and infection, however, does not justify claims that anemia in infancy is commonly "due to infection." In infants infection may, of course, result in anorexia, emesis or diarrhea, and so interfere temporarily with the dietary intake of essential blood-building constituents. Even relatively mild infections may result in transient reduction in the serum iron and diversion of iron to the tissues. It is doubtful, however, whether infection per se does more than aggravate a pre-existing iron-deficient state.

Unlike the anemias associated with infections, the anemia occurring frequently in infants is not hemolytic, is rarely due to blood loss, and

TABLE 19. *Morphologic and Chemical Characteristics of Iron Deficiency Anemia in Infants with or without Infection*

DETERMINATION	WITHOUT INFECTION		WITH INFECTION	
	No.	Mean	No.	Mean
R.B.C. (M./mm.³)	10	4.96	16	4.71
Hgb. (gm.%)	10	8.1	16	7.7
Hematocrit ml./100 ml.	10	29.2	16	27.4
MCV (c.u.)	10	59.1	16	59.4
MCHC (%)	10	27.6	16	27.9
Plasma iron (mcgm. %)	10	32	16	32
T.I.B.C. (mcgm. %)	5	490	13	414
Saturation (%)	5	6.5	13	7.7

differs too in several important respects from the anemia complicating severe chronic infections.[3]

Table 19 shows morphologic and chemical determinations made on 26 anemic infants. Ten of these infants were free of any evidence of infection, while the remaining 16 were suffering from a variety of infections, mainly respiratory and gastroenteric disorders of acute onset and short duration. The figures represent the mean for the various determinations in the two groups of infants. It is immediately apparent that the degree of anemia, as well as the morphologic and chemical characteristics of the anemia in the two groups, is almost identical, and that the features of the anemia are those of iron deficiency, not of infection. The term "anemia of infection" often has been used ill advisedly.

Role of Miscellaneous Factors

Other factors may be operative in the genesis of iron depletion. Certain of these, perhaps important, but as yet incompletely studied, include individual variations in the absorption of iron, the role of family relationships, anxiety, fear and other emotional disturbances, the role of deficiencies of pyridoxine and copper, and so forth.

CLINICAL MANIFESTATIONS

Because the hypoferric anemia of infancy is usually mild and because its onset is usually insidious and its progress slow, compensatory adjustments are often nearly complete. The infant, therefore, exhibits few signs and symptoms directly attributable to the anemia; the finding of the latter comes as a surprise, often being discovered only when some other associated disturbance is being investigated. Even severe degrees of anemia may be well tolerated and may go unrecognized not only by the parents, but by the physician as well. In the survey previously referred to, pallor was seldom noted by the attending doctor or by the house officer when the hemoglobin exceeded 7 gm. per 100 ml. and was appreciated consistently only when the hemoglobin was below 6 gm. Nearly everyone has had the experience of suspecting anemia in a fair-complexioned baby, only to have normal blood values reported by the laboratory. Obviously the converse is true; i.e., pallor of the skin and mucous membranes may not be recognized until severe anemia exists. In the latter circumstance extreme irritability, anorexia and listlessness are frequent. Pica is not at all uncommon.

Few abnormalities are detected on physical examination unless anemia is marked. Then hepatosplenomegaly may be noted in approximately 10 per cent and retardation of growth in about twice this number. Precordial systolic murmurs of variable intensity are frequent and, when coupled with radiographic evidence of cardiac enlargement, also frequent, may be confused with organic heart disease. Edema of the face and eyelids may be a manifestation of hypoproteinemia or may accompany cardiac failure. The latter is uncommon, but does sometimes occur. Changes in the texture of the hair and skin as well as the spoon-nails and atrophy of the tongue noted in some adults are rare in infants. Fever is unusual unless a complicating infection exists.

LABORATORY FEATURES

A reduction in the amount of hemoglobin per red cell and some decrease in size of the erythrocyte are the outstanding features of this type of anemia and are easily appreciated by examination of a peripheral blood smear. The more severe the anemia, the more apparent these changes become. Some of the cells assume oval or pear shapes, and target cells are common. A few erythrocytes well filled with hemoglobin and even larger than normal in size are often seen, but the over-all appearance is one of microcytosis and hypochromia. Polychromatophilia and nucleated red blood cells may be found.

The hemoglobin and hematocrit are reduced to a greater degree than the red cell count. The latter may, in fact, be normal or even somewhat elevated. Hemoglobin concentrations of 6 to 10 gm. and hematocrits of

20 to 30 ml. per 100 ml. are commonly found. In approximately 10 to 15 per cent of patients even lower values may be encountered. We have seen patients with as little as 1.9 gm. of hemoglobin and with the hematocrit as low as 8 per cent packed cell volume.

The disproportionate reduction in hemoglobin and hematocrit leads to a lowering of the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC). It seems worth while emphasizing that unless the quantitative data on which these calculations depend are carefully done, the red cell indices may not be as expected and may, in fact, be misleading. Usual variations for these values are 65 to 75 cubic microns for the mean corpuscular volume, 15 to 20 micromicrograms for the mean corpuscular hemoglobin, and 25 to 30 per cent for the mean corpuscular hemoglobin concentration. Values below 50 for mean corpuscular volume, below 10 for mean corpuscular hemoglobin and below 20 for the mean corpuscular hemoglobin concentration are rare. Guest and co-workers[10] have pointed out that the mean corpuscular volume and the mean corpuscular hemoglobin are the first to fall as anemia develops.

Additional laboratory features of iron deficiency anemia may be found in standard hematological references.[35]

DIFFERENTIAL DIAGNOSIS

The morphologic features of iron deficiency anemia are shared by certain other hematological disorders, such as the Mediterranean or thalassemic syndromes, and some of the newer hemoglobinopathies. Failure to respond to adequate doses of iron may be the first clue to the existence of one of these, although other features, as discussed elsewhere in this volume, usually suggest the correct diagnosis.

A microcytic, hypochromic anemia, also unresponsive to iron, and due to a naturally occurring deficiency of pyridoxine, has recently been described in adults.[14] We have failed to find evidence of a deficiency of this vitamin in a few infants with hypoferric anemia, although others[21] have suggested that a "pyridoxine need" may exist in some. The artificial induction of vitamin B_6 deficiency in one infant did lead to a microcytic anemia.[26]

The exact nature of other reported microcytic hypochromic anemias remains undetermined.[38]

PREVENTION

In preceding paragraphs, evidence was presented indicating that iron deficiency is the commonest cause of anemia in children, that iron depletion exists in many who are "normal" by currently accepted hemato-

logical standards, and that anemia usually cannot be ascribed to a single specific cause, since the total iron content or distribution of iron in a newborn infant cannot be ascertained, the efficiency with which food iron is absorbed can only be estimated, and significant loss of blood at birth may have gone unrecognized. Some means of prevention therefore deserves serious consideration, and should either be adopted as a routine measure or applied according to a particular plan.

A prophylactic program should logically begin with administration of iron to the expectant mother, even though a relation between maternal and infant iron stores has not been established to the satisfaction of all. The need probably increases with each succeeding pregnancy.

As for the infant's care, the physician may feel virtually certain that supplemental iron will be necessary if the infant is prematurely born or is the result of a multiple birth, if the mother was known to have had an untreated iron deficiency anemia during pregnancy, frequent pregnancies or menorrhagia, if a previous sibling had iron deficiency anemia during infancy, if the infant lost blood at birth, or if the infant is "later born" compared with the first born. Under such circumstances and as previously emphasized, it is unsafe to rely upon dietary iron alone, and wise to provide two to three times the usual dose of iron recommended for prophylaxis, i.e., 10 to 15 mg. of elemental iron daily rather than 5 mg. Although there is no agreement on the optimum age at which supplemental iron is utilized most efficiently, iron may be started advantageously at six to eight weeks of age.

Should the physician choose not to recommend iron as a routine procedure, it seems reasonable to suggest that a hemoglobin determination at least be made at five or six months of age and that iron be administered if a value below 10.5 gm. per 100 ml. is recorded. As previously emphasized, anemic infants will go unrecognized if one relies only on visual appraisal of the patient's color.

The prophylactic dose of iron cannot be rigidly defined, since, for reasons previously discussed, an infant's requirements are not accurately known. Doses varying from 5 to 25 mg.[20] of elemental iron per day have been recommended. This rather wide range may be the result of variations in the types of iron salts used by various investigators. A recent study indicates that a small dose of iron (5 mg. per day for infants three to six months of age and 10 mg. daily thereafter) given in the form of a concentrated ferrous sulfate preparation maintains the hemoglobin at about 13 gm. per 100 ml. between the ages of six and 12 months.[20]

TREATMENT

When prophylactic procedures have not been used and iron deficiency anemia develops, treatment with an adequate dose of a suitable

iron salt is indicated. The administration of iron should be considered essential, not merely a useful adjunct to a better planned dietary. Intelligent therapy, of course, includes an attempt to identify the cause. Only occasionally, and usually in an older child, will chronic blood loss, absorptive defects and the like[35] be incriminated. Attention to the underlying cause must then be combined with the administration of iron.

Several factors should influence the physician's choice of the iron preparation to be used. Although an adequate response may be gained with ferric iron, experimental evidence indicates that bivalent salts are better absorbed than trivalent ones.[36] For this reason ferrous salts in the form of the sulfate or gluconate are most widely used. While iron in tablet or capsule form is suitable for the older child, a palatable solution is preferable for the infant. The preparation used must also be selected on the basis of its content of elemental iron. In addition to iron, many available mixtures contain cobalt, molybdenum, copper, manganese and other metals as well as various vitamins and liver fractions. Although some of these play an important role in the metabolism of iron, there is insufficient proof that any one is such a necessary adjunct to therapy as to require its inclusion in an iron preparation. Not only is the amount of elemental iron contained in some of these mixtures inadequate, but the action of certain other constituents is incompletely understood and even potentially dangerous. Furthermore, the cost of some is exorbitant; e.g., to administer 60 mg. of elemental iron in one commercially available preparation costs about three cents per day, while another costs about 50 cents per day, a not inconsiderable difference when it is realized that iron therapy should be continued for two to three months.

A dose which furnishes 60 to 75 mg. of elemental iron per day and is given in three or four divided doses is satisfactory for patients up to three years of age, after which two to three times this amount may be necessary. A minimal effective dose has not been established for children, but 4.0 mg. of iron per kilogram of body weight per day has been suggested.[17] To avoid intolerance, a smaller quantity is given initially and then gradually increased. The juice of citrus fruits makes an ideal vehicle for iron therapy in infants not only because the metallic taste is disguised, but also because ascorbic acid enhances the absorption of iron.[19] Iron is also well tolerated and effective when given in milk, though possibly less well absorbed.

A stable saccharated oxide of iron, administered intravenously, has been available for the past few years, and a dextran-iron compound for intramuscular use, now being given clinical trials, will probably soon become generally available. Parenteral iron should be reserved for those iron-deficient children who are unable to derive the full benefit

from orally administered iron or who cannot tolerate iron given by mouth, e.g., patients with celiac disease or ulcerative colitis, and for those whose parents fail to cooperate in the administration of oral iron. Parenteral iron therapy also offers a more dependable, and perhaps more rapid, means of preparing the iron-deficient child for elective surgery.

The correct dose to be given must be calculated from knowledge of the patient's approximate blood volume (75 ml. per kilogram of body weight is a suitable estimate), his hemoglobin deficit and the iron content of hemoglobin (0.34 per cent). A convenient and simplified formula for calculating the amount of iron required to alleviate the anemia is as follows:

Body weight in kg. $\times$ (13.5 —hgb. in gm. %) $\times$ 2.5 = mg. iron needed.

Because this figure does not take into account the depletion of iron stores, an additional 10 to 15 per cent may be given.

In older children, as in adults, the required amount of iron may be administered in doses of 40 to 100 mg. per injection at intervals of a few days to a few weeks. In hospitalized infants, in whom repeated venipunctures are troublesome, we have found it convenient first to start an intravenous drip with a clear solution and to administer the iron either through the tubing or a three-way stopcock at two to three hour intervals and in doses of 20 to 40 mg. until the total calculated dose has been given. Others have successfully given larger single doses (10 mg. per kilogram) at intervals of a few days.[12]

The danger of administering amounts of iron in excess of the body's needs warns against the indiscriminate use of such compounds. In addition, parenteral therapy is expensive, troublesome to administer, may cause pain and inflammation at the site of injection and may be associated in 5 or 10 per cent of patients with undesirable systemic reactions, e.g., fever, tachycardia, pallor or flushing of the skin, perspiration, and nausea and vomiting. Discoloration of the skin due to iron deposition has been noted even at sites distant from the point of injection.

When the correct diagnosis has been made and an adequate dose of iron given, a characteristic therapeutic response occurs. Often before any of the laboratory data change, the infant's behavior improves and he spontaneously accepts previously rejected foods in quantities which surprise and gratify the parents.

The percentage of reticulocytes increases three to five days after the institution of iron and reaches a maximum in five to ten days. The magnitude of this response is inversely related to the initial hemoglobin level and is usually greater in patients given intravenous iron.

As it is not always convenient to perform reticulocyte counts frequently and since, in fact, reticulocytosis may be slight when the ane-

mia is mild, the effectiveness of therapy must be gauged by responses in hemoglobin, hematocrit or red cell count. A change in the number of erythrocytes is the least reliable guide in estimating the adequacy of response, since the red cell count may be normal or high initially and, if high, may fall during therapy.

The hemoglobin, after a lag of three to four days, usually rises at the rate of 0.15 to 0.3 gm. per 100 ml. per day until normal values are approached, after which the rate of regeneration slows. The increase in

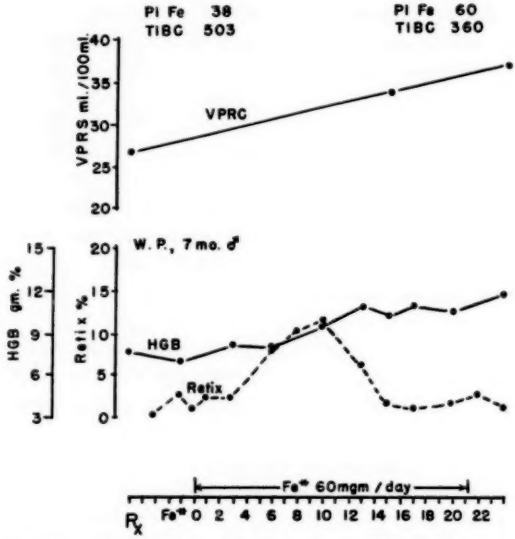

Fig. 31. Hematological response to iron administered orally.

hemoglobin may be somewhat faster when iron is given intravenously rather than orally, although the rate of recovery in identical twins to these two forms of iron was strikingly similar.[5] If a satisfactory hemoglobin rise has not occurred within three to four weeks, one of several possible explanations for failure should be sought.

The commonest cause of failure to respond is inadequate dosage of iron, arising either because the prescription contained too little iron or because the amount prescribed was not administered by the parent. Additional causes include incorrect diagnosis, i.e., confusion with other microcytic hypochromic anemias not responsive to iron therapy, defective absorption, or blood loss in excess of that compensated for by the administration of iron. Severe, chronic infections interfere with the utilization of iron. However, the infections commonly complicating hypoferric anemia in infants are usually not of this type and do not

influence significantly the response to iron therapy. The reticulocytosis and hemoglobin generation shown in Figure 31 occurred in an infant with fever and radiographic evidence of bronchopneumonia. Similar or better responses can be duplicated many times from our case records of infants with other acute infections complicating iron deficiency anemia.

The transfusion of whole blood is seldom required, and is to be discouraged except in those few instances when the severity of a complicating illness necessitates the immediate support provided by blood. As previously noted, compensatory physiological adjustments to the slowly developing anemia may be nearly complete. The decision as to whether or not blood is to be given should be determined by evaluation of the patient's general condition rather than by an arbitrary level of hemoglobin. Many patients with as little as 2 to 3 gm. of hemoglobin per 100 ml. have been successfully treated with iron. Transfusions are not only expensive, uncomfortable and difficult to administer, but also potentially dangerous, since undesirable sensitization may result and circulatory overload may lead to cardiac failure and death. Should cardiac failure exist, the slow administration of packed red cells combined with digitalization is recommended.

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THE ACUTE LEUKEMIAS OF CHILDHOOD

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Childhood leukemia presents one of the most impelling problems in the practice of pediatrics today. Its peculiar age distribution, with a peak incidence within the first four years of life, the unknown factors which initiate the leukemic state, its prominence as a leading cause of death in childhood and its universally fatal course in spite of spontaneous or induced remissions, continue to baffle the clinical and research investigators. By definition, leukemia in childhood is predominantly an acute disease of unknown etiology, whose protean symptomatology is dependent upon the effects of the leukemic transformation of normal blood leukocytes or their precursors into cells which grow without restraint both within and outside the blood-forming organs. The modern concept of leukemia accepts the premise that nonspecific genetic influences acting in conjunction with unknown external factors combine to cause the leukemic transformation. The therapeutic regimens which have brought about the striking remissions in the disease are directed to the eradication of the leukemic cells by application of chemotherapeutic principles learned through the methods of controlling bacterial growth and recently applied to the inhibition of growth of malignant cells.

In the past eight years, which represent the era of chemotherapy of leukemia, the acceleration of interest in the field of leukemia research has been rapid, and the clinical management of the leukemic child has been improved greatly. In this review an attempt will be made to discuss some of the research and clinical observations which have a direct bearing upon the management of the child with the disease and the parent who faces it.

EPIDEMIOLOGY

The incidence of leukemia is higher in children under the age of four years than at any other age during childhood. In his analysis of 1500 cases of childhood leukemia in 1942 Cooke[16] found that 55.4 per cent of the cases occurred in the age group 0 to four years, with a peak incidence at three years. More recent surveys[19, 32, 39] confirm the observation, as do the author's 232 cases, 56 per cent of which were under four years of age. After the age of four years the incidence curve

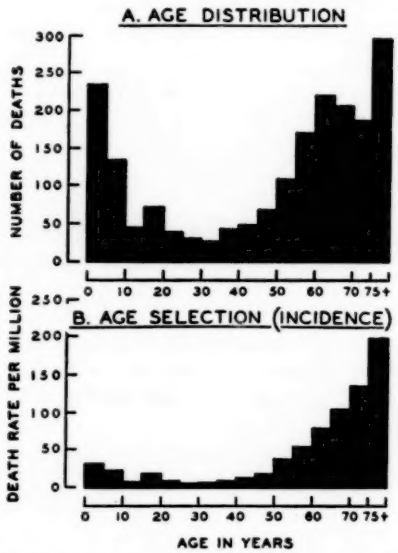


Fig. 32. Age distribution and age selection (incidence) at death from lymphatic leukemia for white males in the United States during 1949. (By permission from Alexander G. Gilliam: Age, Sex and Race Selection at Death from Leukemia and the Lymphomas. *Blood*, 8:693–702, 1953.)

falls (Fig. 32) gradually to age 10 to 14, rises again between 15 and 19 years, then falls again, not to rise sharply until age 40 to 44 years.

It is questionable whether the incidence of leukemia in children is increasing or not. The death rate from leukemia at all ages has increased in the past 20 years, and it is more notable in children under the age of five years.[69, 77] Cooke[18] compared the United States mortality statistics for the years 1930, 1940 and 1949 and found that the steepest rises in the curve of the death rates plotted against the age at death were between the ages 0 and 14 years and 55 and 60 years. In the youngest childhood age group, 0 to four years, the rate is from 2.2 to 5.1 per 100,000 deaths between 1930 and 1949. Whether the rise in

incidence is real or apparent is debatable. Prior to 1939 the cases of aleukemic leukemia were not included in the leukemia statistics; the inclusion of these cases might account for the increased rate since 1939, but since the most striking rise occurred in the decade 1930–9, another explanation must be sought to account for the rapid rise during that decade. Improvement in accuracy of diagnosis is no doubt an important factor, for probably many cases were recognized as leukemia which had previously been accepted as a leukemoid response to infection, von Jaksch's anemia, and others. However, the majority of the cases seen during the 1930's were diagnosed by clinical and peripheral blood findings, and it is only during the past 15 years that bone marrow aspirations have been in general use; even with the aid of marrow examinations which detect the aleukemic type, the rising curve of incidence was not as steep during the decade 1940–9 as in the previous decade.

Although the increase in the total number of cases is not of such magnitude that it can be concluded that leukemia is increasing among children, it is clear that more of them are admitted to children's hospitals throughout the country for intensive care. The tendency for these cases to aggregate in the teaching centers invalidates the use of statistics from hospital admissions for statistical evaluation of incidence.

ETIOLOGY

The role of *heredity* in childhood leukemia is dubious. In most cases the family history is negative for the occurrence of leukemia in a close relative. The occurrence of leukemia in relatives of patients with leukemia was investigated by Vidabaech,[88] who conducted an extensive genealogical study in Denmark, where it was possible to trace the family histories on 209 families of leukemic patients and a similar number of nonleukemic controls; he found that leukemia was diagnosed in 17 of 209 families of leukemic patients and in only one of 200 families of the control group. The genetic pattern of inheritance did not reveal a specific trait; the factor was not sex-linked and did not fulfill the characteristics of a recessive gene. He concluded that leukemia seems to be dependent upon, among other things, a nonspecific hereditary predisposition to cancer.

There are occasional instances of the disease in siblings or twins; Anderson[1] reported an extraordinary family in which five of eight siblings died of leukemia before the age of eight years. Ward[89] reported a case of leukemia in a 21 month old child whose cousin died of leukemia at age three months; the child's aunt had died at age 26 years of acute leukemia, and there were no cases of the disease in her descendants through two generations. Among 23 cases in the author's series there were two instances of familial leukemia; in one family two of seven

siblings died of acute stem cell leukemia before the age of four years, and a paternal uncle died of acute lymphatic leukemia six weeks before the birth of his first child, a boy who had acute lymphoblastic leukemia at age two years. The occurrence of leukemia in twins during childhood has been recorded in seven instances. In five sets[2, 17] both twins were affected, and four of the five were identical twins (uniovular). In one set[17] of identical twins, only one twin was affected. Cooke[17] reported one set of fraternal twins, both of whom had leukemia at the ages of 18 and 24 months. The literature is an incomplete record of the number of instances of leukemia occurring in only one of a set of twins.

These instances of familial leukemia provide strong evidence that hereditary factors play an essential role in human leukemia, as they do in experimental animals in which inbreeding of certain strains enhances the incidence of the disease.

Congenital leukemia has been reported in 20 cases,[4, 53] and the author has seen two more; it seems likely that it is more common than the number of recorded cases indicates. The association of other congenital defects—Mongolism,[4, 53] cardiac defects, abnormalities of the bones, and so on—with congenital leukemia suggests that an intrauterine disturbance occurs between the sixth and ninth weeks of gestation[49] and initiates the leukemic process in the fetus. There is no direct evidence that leukemia is transmitted from mother to child. The author knows of no report of the occurrence of leukemia in a child whose mother suffered from leukemia during pregnancy, although there are several reports[91] of healthy children born of leukemic mothers.

The *virus* etiology of human leukemia is unproved. The virus nature of avian leukosis, which was suggested by the work of Engelbreth-Holm[29] in 1938, has been confirmed in several laboratories, and virus particles in the plasma of chicks with avian leukosis transmit the disease to other chicks.[25] The virus etiology of mouse leukemia is still unproved, although the investigations in this field have been stimulated by the observation of Gross and his co-workers. Gross[36] demonstrated that tissue extracts of AK leukemic mice inoculated into one day old C3H (low incidence strain) mice induced leukemia at the age of about 10 months in about 80 per cent of the mice. These results were duplicated with cell-free filtrates of the leukemia tissues,[37] and it was shown also that the induced leukemia was transmitted to the offspring of the inoculated animals.[38] Recently the observations of Schwartz[74] have brought further interest to this field; in a strain of AKR mice, who normally have leukemia spontaneously in a high percentage of animals after the age of five to seven months, Schwartz noted that animals inoculated at the age of four to 12 weeks intrathecally with a cell-free extract of leukemic AKR mouse brain had leukemia within three to four weeks after inoculation. A similar acceleration of mouse leukemia occurred when human leukemic brain extracts were used. The nature of the leukemogenic agent which induces or accelerates the mouse leukemia is not determined, but this is the particular direction of the experimental studies at present.

The role of *infections* as a predisposing factor in childhood leukemia is unknown. It is common, indeed, to obtain a history of onset which indicates that a child has been well until the occurrence of a series of common and minor infectious illnesses, after which the symptoms and

signs suggestive of leukemia appear. But until it can be shown that nonspecific infections are concerned with the transformation of normal into leukemic cells, it is perhaps unprofitable to speculate on this point. However, it seems possible that the role of infections as a predisposing cause may be related to an immunological mechanism which affects the hematopoietic system adversely.

Within the past decade techniques have been developed for the separation of blood leukocytes, which, together with advances in the fields of immunology and hematology, have contributed greatly to our knowledge of the immunological properties of leukocytes and the detection of antileukocyte antibodies in plasma. Antigenic differences in normal leukocytes obtained from various persons have been detected. Dausset,[20] using leukoagglutinating antiserums obtained from patients after multiple transfusions or from patients whose titer for leukoagglutination is high with known cause, described antigenic differences in the leukocytes which are unrelated to the blood group of the erythrocytic series, although leukocytes do contain A or B substance. Specific antigenic differences in human leukemic cells, as compared with leukocytes from normal persons, have not been demonstrated, although Thompson[85] presented evidence of the specific antigenetic property of the leukemic cells of mouse leukemia. The evaluation of the data accumulating rapidly in the field of immunohematology must await further developments.

Of the external factors which combine with genetic influences to predispose to leukemia in childhood, less is known than for adult leukemia. *Radiation* is strongly incriminated as a leukemogenic agent in adults by the various reports of the high incidence in radiologists[58] and in victims of the atomic bombs.[59] Similarly incriminating evidence is suggested by the recent survey by Simpson et al.,[78] who compared the incidence of leukemia in children who received radiation to the mediastinum for enlarged thymus in infancy with the incidence in their nonirradiated siblings; they found that leukemia developed in six of 1400 irradiated children, but no cases occurred in their 1765 siblings. Since the approximate incidence of leukemia is one among 2000 children, these figures indicate a high statistical difference in the incidence in the irradiated children, and is sufficient warning to the clinician to avoid unnecessary radiation, particularly to the young infant. The data are particularly disturbing in regard to the lack of apparent relationship between the therapeutic doses given and the incidence of leukemia; the incidence was as high among children who received less than 200 roentgens as among those who received a higher dose. It is perhaps significant that the majority of these children were treated as young infants for respiratory distress, presumably due to thymic hypertrophy, and it is possible that genetic and constitutional factors influ-

enced their susceptibility to radiation damage. To the author's knowledge, there are no data available on the relative incidence of leukemia in children who presented x-ray evidence of mediastinal lymphatic enlargement in infancy as compared with a group of children whose chest findings were within limits of normal roentgenologically. On the other hand, careful inquiry into the history of exposure to radiation during fetal life or early infancy in our group of leukemic children has failed to reveal any evidence that previous radiation could be incriminated as the etiological factor in childhood leukemia.

TYPES

Among the 1000 cases of acute leukemia which Tivey[86] collected from the literature, 445 cases were in children under 15 years of age. The percentage distribution of the various cytological types was as follows: lymphocytic, 78 per cent; myelocytic, 19.7 per cent; monocytic, 4.9 per cent. The high percentage of the lymphocytic type corresponds

TABLE 20. *Cytological Types of Leukemia in Children**

DIAGNOSIS	NUMBER OF CASES	PERCENTAGE
Stem cell	195	83.9
Myeloblastic (promyelocytic)	14	6.06
Monoblastic	12	5.15
Lymphosarcoma (or leukosarcoma)	9	3.8
Reticuloendothelial	2	0.86

* 232 cases treated between 1946 and 1956, Department of Pediatrics, University of Chicago.

to that of the undifferentiated stem cell leukemias in our series of 232 cases (Table 20).

SYMPTOMS AND SIGNS

The symptomatology and course of childhood leukemia are similar in all the acute blastic forms regardless of the cytological type. The first symptoms which date the onset of the disease commonly appear after a period of vague malaise which has followed a common upper respiratory infection or contagious disease. A low grade fever, pallor and a tendency to bruise are common symptoms which lead the parents to consult the physician, who can often establish the diagnosis promptly by a blood cell count. In the fulminating form the onset is abrupt, simulating an acute infectious disease with rapidly developing signs of anemia, purpura and organ involvement. In roughly one third of the cases, rheumatic fever has been suspected because of swelling of the joints of the hands or feet or pain in the knees or hips. Pain in the

bones may be severe even before x-ray evidence of leukemic changes is detectable. Dyspnea is a common first symptom in cases with mediastinal lymph node enlargement; in the leukosarcomas the primary involvement of the mediastinal nodes is characteristic. The early signs of the disease are related to the anemia, purpura and enlargement of the hematopoietic organs. Splenomegaly occurs regularly, although not to an impressive degree in the early phase of the disease, and the peripheral lymphadenopathy may also be minimal at a time when the blood findings are diagnostic.

The pathognomonic sign of leukemia is the presence of a large percentage of blast forms in the blood and marrow. In the majority of cases the peripheral blood findings are diagnostic. The total leukocyte count varies, leukopenia being as common as a hyperleukocytosis. If the total number of circulating blasts is elevated, the smear is diagnostic; if a leukopenia exists, examination of the marrow is necessary for the detection of the blast forms. The anemia is normochromic and, rarely, hemolytic; it progresses rapidly as the degree of leukemic transformation proceeds. The platelet count is usually severely depressed, especially in the stem cell and myeloblastic types, and to a lesser degree in the monoblastic types; the thrombocytopenia is the dominating factor in the mechanism of the hemorrhagic tendency throughout the disease.

In most cases the cytology of the bone marrow is pathognomonic of the leukemic transformation. During the early stage of the disease the predominating cell type is present in excessive numbers, and normal myelopoiesis is depressed. In rare instances the marrow pattern is still within normal limits; granulopoiesis and erythropoiesis are then active, and the sum of blast forms and lymphocytes in the range of 30 per cent. The diagnosis may rest on the morphology of the blast forms. In such an instance a definite diagnosis can be determined only by examinations after an interval of a few days or weeks. The diagnosis may be particularly difficult if the marrow is aplastic and the aspiration yields only a few cells, although a careful preparation which includes particles of marrow tissue will usually reveal the presence of the typical blasts in a marrow which is otherwise hypoplastic.

The manifestations of the later stages of the disease are protean, and a full discussion will not be attempted in this review, except for a brief mention of the complications which are particularly resistant to therapy.

COMPLICATIONS

The hemorrhagic manifestations of the secondary type of thrombocytopenic purpura are a mortal threat throughout the course of the disease and a common cause of death in the fulminating type. Intracranial

hemorrhages are not infrequent, particularly if the total number of circulating blasts is high. The hemorrhagic tendency is aggravated by infections which produce local areas of hyperemia and further damage to the vascular endothelium; bleeding into the oral and nasopharyngeal cavity is one of the distressing features of the disease. Massive bleeding into the gastrointestinal tract occurs as a result of agranulocytic ulceration of the bowel. When the intactness of the mucocutaneous surfaces of the body orifices is broken, painful areas of necrosis and hemorrhage develop. The hemorrhagic signs improve and disappear as remissions occur, but until the remission is induced they require active treatment with whole blood or platelet-containing fractions of whole blood.

The agranulocytosis which occurs concomitantly with the leukemic transformation of the blood-forming organs is probably the main factor in the increased susceptibility to infection. Infection and septicemia due to a variety of organisms were common contributory causes of death in leukemias treated in the preantibiotic era, and the shortness of the life span in this era was due largely to these complicating infections. At present the infections which offer the serious complications are those due to the resistant strains of *Staphylococcus aureus, Bacillus proteus* and the monilial infections. In order to minimize the development of resistant bacterial strains, it is our practice to use antimicrobial drugs only as indicated by signs of infection and under bacteriological control. Prophylactic antibiotic therapy is avoided as much as possible.

Renal infiltration not infrequently complicates the administration of an antileukemic drug by restricting renal function. Rapid destruction of the leukemic blasts, resulting in a rapid breakdown of nuclear protein, increases the excretion of urates. If the renal clearance is reduced by the extent of the leukemic infiltration, signs of impending uremia develop with elevated blood levels of nonprotein nitrogen and uric acid. Furthermore, the early development of renal infiltrates resulting in large palpable kidneys has proved, in our experience, to be an ominous prognostic sign, for eradication of these infiltrates appears to be particularly difficult to achieve.

Infiltrations into the central nervous system also are particularly difficult to treat. The neurological manifestations of the disease are more frequent now that a prolongation of life has been achieved, although they are similar to those which occur in the untreated cases. The increased frequency of leukemic meningitis in patients treated with the antileukemic drugs, and whose hematological remission is sustained, has caused concern regarding the lack of effectiveness of the drugs against intracranial infiltrates; there is no proof that the drugs predispose to neurological infiltration. The symptoms and signs indicative of leukemic meningitis are those of progressive intracranial hypertension, with headaches, recurrent vomiting, papilledema and later x-ray evi-

dence of separation of the cranial sutures in younger children. The cerebrospinal fluid is clear and under increased pressure, the protein content is moderately elevated, the sugar content normal and the cell count elevated, ranging from 300 to 1500 cells, the majority of which can be recognized as blast forms. The symptoms and signs regress after x-ray treatment to the skull, but recurrence or progression of the infiltration is inevitable.

DIFFERENTIAL DIAGNOSIS

In the differential diagnosis of early leukemia the importance of a variety of conditions depends upon the presenting symptoms and signs of the case in point.

Acute lymphocytosis, which is characterized by a benign lymphocytosis occurring in epidemics in young children without signs of illness, splenomegaly or purpura, may cause concern if it is complicated by a nutritional anemia. The morphology of the lymphocytes remains normal, although the total leukocyte count is commonly in the range of 20,000 to 40,000 and may reach 300,000.

Infectious mononucleosis with predominant signs of lymphadenopathy and splenomegaly usually exhibits other signs of the acute infectious disease, such as angina and rash; the absence of a significant degree of anemia or thrombocytopenia and the presence of the typical basophilic lymphocytes of the Downey type confirm the clinical diagnosis. In occasional cases the morphology of the lymphocytes is atypical, and a marrow examination is required to exclude a leukemia of the monoblastic type.

Rheumatic fever offers a particularly difficult differential problem since the symptoms and signs of leukemic arthritis may simulate rheumatic arthritis of the hands, feet or hips. The low grade fever and progressive anemia are common to both; the absence of a hemorrhagic tendency, the positive antistreptolysin titer and/or the signs of rheumatic carditis, the response to salicylates and the absence of abnormal cells in the peripheral blood will usually establish the diagnosis, although in some cases a marrow study is necessary.

Rheumatoid arthritis with mild joint signs, regional lymphadenopathy, splenomegaly, anemia and lymphocytosis offers a similar problem, for in the typical case of Still's disease the clinical picture is typical, and in occasional cases of leukemia the same signs are present.

Disseminated lupus erythematosus in its early stages runs a course similar of that of leukemia, although its later manifestations in the skin and collagen tissues are characteristically different. The presence of large numbers of typical lupus cells in the blood and marrow is diagnostic.

Acute thrombocytopenic purpura in children produces signs of petechial and purpuric bleeding into the skin, as in leukemia, but severe anemia is absent unless frank hemorrhage occurs. The marrow pattern of granulocytopoiesis and erythropoiesis is normal, and megakaryocytes are present in excess numbers, although platelet formation in their cytoplasm is lacking.

Acute aplastic anemia can be excluded only by examination of the bone marrow, which shows a general depletion of all the cellular elements of the marrow.

Septicemias due to the common pyogenic organism, or meningococcus, which are accompanied by purpura are simulated by the acute fulminating leukemias, and can be excluded only by means of a blood culture.

Hodgkin's disease is differentiated by the preferential unilateral enlargement of the cervical nodes, the characteristic fever and the absence of severe anemia or purpura in the early stages.

Lymphosarcoma in a child is potentially capable of disseminating to a leukemic process, which can be detected in the marrow prior to the release of the leukemic cells into the peripheral blood.

Reticulocytosis closely simulates the more subacute forms of leukemia and is excluded by the marrow findings.

Histoplasmosis of the systemic type produces the general clinical findings of a septicemia accompanied by splenomegaly and a granulocytosis with severe secondary anemia and thrombocytopenia. The findings of Histoplasma by blood or marrow cultures or directly in the phagocytic cells of the marrow is diagnostic.

NATURAL COURSE

The natural course of untreated childhood leukemia is seldom seen in modern times. The disease is rapidly fatal in all forms without the use of blood transfusions and antimicrobial therapy. The modifying effect of transfusions is seen not only in the symptomatic improvement of the patient, but also in the occasional remission which follows the use of several transfusions or exchange transfusions. Antimicrobial drug therapy which controls secondary infection prolongs the survival time to some extent. Spontaneous remissions without transfusion therapy are rare, indeed. In Southam's review[83] of the natural history of the disease in 150 cases seen at Memorial Center between 1926 and 1940, one of 105 children in the series had a spontaneous complete remission on a supportive therapeutic program which included transfusions. In our 55 cases treated between 1930 and 1941 with a similar regimen, only one child showed a complete hematological remission, which lasted for three months; this occurred after her first transfusion. Infections,

particularly if severe and associated with fever, have produced remissions, and the author has seen a few instances of this effect following severe pyogenic infection such as peritonitis or a local abscess. But aside from the rare remission following such an infection (see Fig. 33), the unfavorable effect of bacterial and viral infections on the course of the disease is impressive. It seems probable that the leukemic child who still retains the natural reserves which enable him to react to severe infection with the normal defense mechanisms is able to control his leukemic process for a limited time.

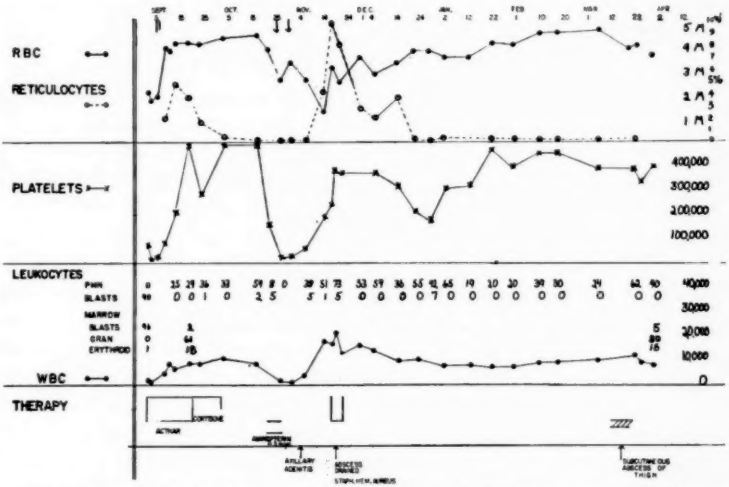


Fig. 33. Remission due to a purulent infection in a child with stem cell leukemia under treatment with ACTH. The ACTH remission was incomplete. The development of an abscess due to hemolytic staphylococcus resulted in a complete remission which lasted for 4 months.

The average duration of life in leukemic children treated before 1948, which dates the beginning of the modern chemotherapeutic regimens for leukemia, was about four and a half months. In an analysis of 40 case records of leukemic children treated at Children's Memorial Hospital between 1916 and 1940 the author found that in 23 children who received no transfusion, and in 17 children who received only one transfusion, the average duration after onset of the first symptom of the disease was 5.1 weeks, with a range of one to 12 weeks. In 55 patients known to the author who received multiple transfusions as the only treatment, the average duration of life was 15.8 weeks, with a range of two to 36 weeks. These latter figures are similar to those reported by Southam for 105 children aged 0 to 14 years treated between 1926 and 1948, in whom the mean survival time was 17.6 weeks. These figures

provide a control series for comparison with the results obtained by the modern therapeutic regimens.

TREATMENT

In the following discussion of the treatment of leukemia in children an attempt will be made to review briefly the experimental investigations which have contributed directly to the clinical management of the disease and to summarize our own results obtained with the therapeutic regimens used currently.

The specific aim of the modern therapeutic regimens for the treatment of childhood leukemia is the eradication of the leukemic cells which permits recovery of the normal process of hematopoiesis. Supportive measures utilizing blood and plasma fractions to control anemia and a hemorrhagic tendency as well as specific chemotherapeutic and antibiotic treatment for intercurrent infection are necessary adjuvants of the clinical management which require evaluation in the assessment of the response to the specific antileukemic drug used.

In addition, and perhaps more important, is the necessity for recognizing the need for a hospital and out-patient environment which lessens the tensions created in the child and in the parents by a long and difficult course of treatment, which requires frequent visits to the hospital. The awareness of the hospital personnel of the emotional stress within the family involved is an essential factor in achieving a measure of adjustment to the total problem; living-in arrangements for the mother may be advisable; financial assistance is frequently necessary; frequent consultations between the medical personnel and parents are essential.

The remissions obtained by a given regimen are conveniently classified as complete, incomplete and failures; the criteria for each have been recently defined by the Clinical Panel of the Cancer Chemotherapeutic National Service Center in an attempt to standardize the terms and permit a comparison of the results obtained by different clinical investigators using the same therapeutic programs. In general, a *complete remission* requires freedom from clinical signs and symptoms of the disease and blood and marrow findings within normal limits for the age of the child; the blasts in the marrow should not exceed 10 per cent, lymphocytes should be less than 20 per cent, and no cells identifiable as leukemic stem cells are permitted. An *incomplete* marrow *remission* allows a total of 70 per cent lymphocytes and blasts in the marrow, with 30 per cent of normal myeloid elements. Blood findings in which hemoglobin is less than 11 gm., the granulocytes less than 1500 per milliliter and platelet levels of less than 100,000 are classified as incomplete remission or failure, according to the values. A *failure* is

defined by the lack of improvement in either the hematological or clinical aspects of the disease, i.e., persistence of leukemic cells in the blood and marrow, anemia, thrombocytopenia and granulocytopenia, with persistent signs of organ enlargement and/or other manifestations of the disease.

The antileukemic drugs which have yielded remissions in the leukemias of childhood became available in the following chronological sequence: the folic acid antagonist drugs Aminopterin and Methotrexate, 1948; the adrenocortical hormones and steroids, 1950; and the purine antagonists 6-mercaptopurine and Azaserine, 1952 and 1953.

In the following discussion hormone therapy will be discussed first, since it is the therapy of choice for the most acutely ill child.

Hormone Therapy

Mode of Action. *The Relationship of the Adrenocortical Hormones to the Lymphatic System.* Hormone therapy of leukemia began in 1950, when potent extracts of the adrenocortical hormones (see Fig. 34) became available, but the rationale for the use of these extracts in leukemia was grounded in the experimental observations of Selye in 1936.

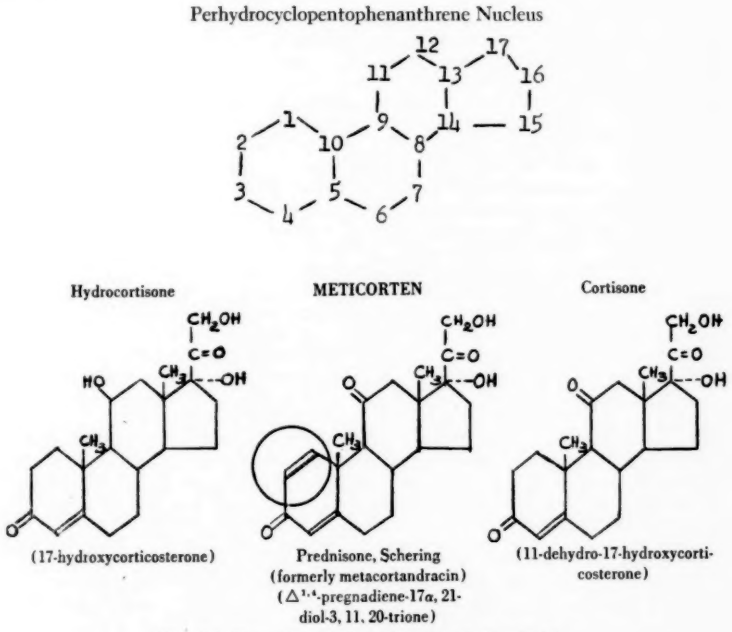


Fig. 34. Formulas of the adrenocortical steroids.

Selye[75] demonstrated that thymus atrophy occurred in rats after the administration of adrenocortical extracts, and this finding was confirmed by numerous investigators. The reviews by Selye,[76] 1946, Valentine, Craddoch and Lawrence,[87] 1948, and Dougherty,[24] 1952, summarize the experimental evidence that augmentation of the adrenocortical hormones results in involution of the thymus, and causes a fall in the level of circulating lymphocytes and eosinophils, and a rise in the level of neutrophilic leukocytes. Reports of studies on the blood picture in the adrenocortical disorders in man indicated that in Cushing's disease the total neutrophile count was elevated and the total lymphocyte count depressed,[21] while in Addison's disease there was a tendency to lymphocytosis and neutropenia.[3] The hematological response to the administration of ACTH in man, as reported first by Hills et al.,[45] 1948, is now well known; within four hours after a single intramuscular injection of 25 gm. of ACTH the characteristic rise in the number of circulating neutrophils and fall in the lymphocytes and eosinophils occurs. On prolonged administration of the hormones the neutrophilia persists and, although the lymphocyte level may rise after the first fall, it remains at a lymphopenic level throughout the period of administration of the hormone.

As steroid chemistry has advanced, methodical studies of the lymphotrophic effect of newly isolated steroids have been reported. In 1952 Money[60] compared the effect of several steroids on the lymphatic organs of intact rats and found that ACTH, cortisone and compound A were the only steroids that produced atrophy of lymph nodes as well as of the thymus. These observations were confirmed by Dougherty and Santisteban,* who, using adrenalectomized rats, established a quantitative relationship between the dose and type of steroid given. Their studies suggested that the C-11 hydroxycorticosteroids were more potent than the C-17 hydroxycorticosteroids in their suppressive effects of the lymphatic tissues.

There is considerable evidence that the lymphopenia and atrophy of the lymphatic tissues are due to cell destruction as well as to a suppression of cell production. The rapid loss of weight of the lymphatic organs, the histological evidence of disappearance of large numbers of lymphocytes, the increased number of fixed and free macrophages throughout the reticuloendothelial system, observations of degenerative changes in lymphocytes in vivo and in vitro, both in cell suspensions[31, 72] and in tissue cultures,[43] present evidence that the adrenocortical hormones are lymphocytolitic. Suppression of mitosis is probably the mechanism by which sustained lymphopenia is maintained.

The experimental indications that the adrenocortical hormones would prove effective in controlling acute leukemia were suggested by the experiments of Murphy and Sturm,[61] (1943) who demonstrated that the longevity of rats with transmissible leukemia was prolonged by administration of desoxycorticosterone, and of Law and Speirs,[54] (1947) who reported marked palliative improvement after intraperitoneal injection of Upjohn's aqueous or lipoadrenocortical extracts in two strains of mouse lymphatic leukemia, C58 and RIL, associated with a sharp fall in the total blast count in the blood and regression of lymphatic organs with histological evidence of degenerative changes, pyknosis, dissolution and depletion of lymphocytes and lymphoblasts in the lymphatic organs. The more recent work in mouse leukemia has yielded conflicting data regarding the effect of the hormones on the survival times of leukemic mice. Levin,[57] 1950, could demonstrate no improvement in the course of AK4 transmissible leukemia by the daily administration of cortisone, and Bloch[8] failed to note any improvement in the survival of mice with methyl cholanthrene-induced leukemia under treatment with 0.1 mg. of ACTH T.D. Odell† observed an increased survival time in transmissible L4946 with cortisone. The improvement in the life span of the treated animals as compared with the untreated animals was due to the prevention of the development of leukemia at the early age typical of the strain.

CLINICAL REMISSIONS WITH HORMONE THERAPY. The antileukemic effect of the adrenocortical hormones in childhood was summarized by Farber[30] in 1951 and reported by several investigators.[6, 66, 73, 84, 92]

* Unpublished observations.

† William Odell: Personal communication.

In our center, where we use a sequential type of therapy with the objective of achieving a prolongation of life, utilizing the different antileukemic drugs in sequence as long as they are effective, we elect to use the hormone regimen for the initial treatment if the child shows signs of acute illness. The broad criteria for evaluating the severity of disease at the time of admission are as follows:

CRITERIA FOR CLASSIFICATION OF SEVERITY OF DISEASE ON ADMISSION

Mildly ill	Minimal clinical signs of disease
	Mild anemia
	Moderate reduction of blood platelets
	Low percentage of blasts in the peripheral blood and marrow
Moderately ill	Moderate clinical symptoms and signs
	Moderate degree of anemia and thrombocytopenia
	High percentage of blasts in the blood and marrow, suppression of granulopoiesis and erythropoiesis
Severely ill	General signs of illness
	Definite signs of organ involvement
	Hemorrhagic manifestations
	Severe anemia, thrombocytopenia and blasts in the peripheral blood
	Replacement of marrow with leukemic cells

The selection of the adrenocortical hormones for the first antileukemic treatment was based upon our experience with our first series of cases treated in 1950–2; in this series of 37 cases complete marrow and clinical remissions were obtained in 75.67 per cent of cases treated. In other series treated initially with the antifolic drugs or with 6-mercaptopurine this high remission rate has not been attained. In subsequent years the remission rate with ACTH has been lower, but still higher than attained by the chemotherapeutic regimens.

The characteristic pattern of response to successful hormone therapy is initiated by prompt improvement in the predominating symptoms of leukemia such as dyspnea due to enlarged mediastinal nodes, or bone pain and fever, followed within five to seven days by notable regression of the enlarged peripheral nodes and spleen and by a gradual fall in the number of circulating blasts. About this time a rise in the reticulocyte count begins, which precedes the recovery of granulocytes or blood platelets, and an examination of the marrow reveals a marked increase in the percentage of erythroblasts even though the recovery of the granulocytes and megakaryocytes is unsatisfactory and the percentage of blasts may be 30 to 50 per cent of the total number of marrow cells. As remission proceeds, the clinical signs and symptoms recede and disappear, and the sign of marrow recovery is completed within two to three weeks of treatment (Fig. 35).

The duration of the remission to hormone therapy is short in most cases. In our first series of 37 cases, treated with intermittent courses,

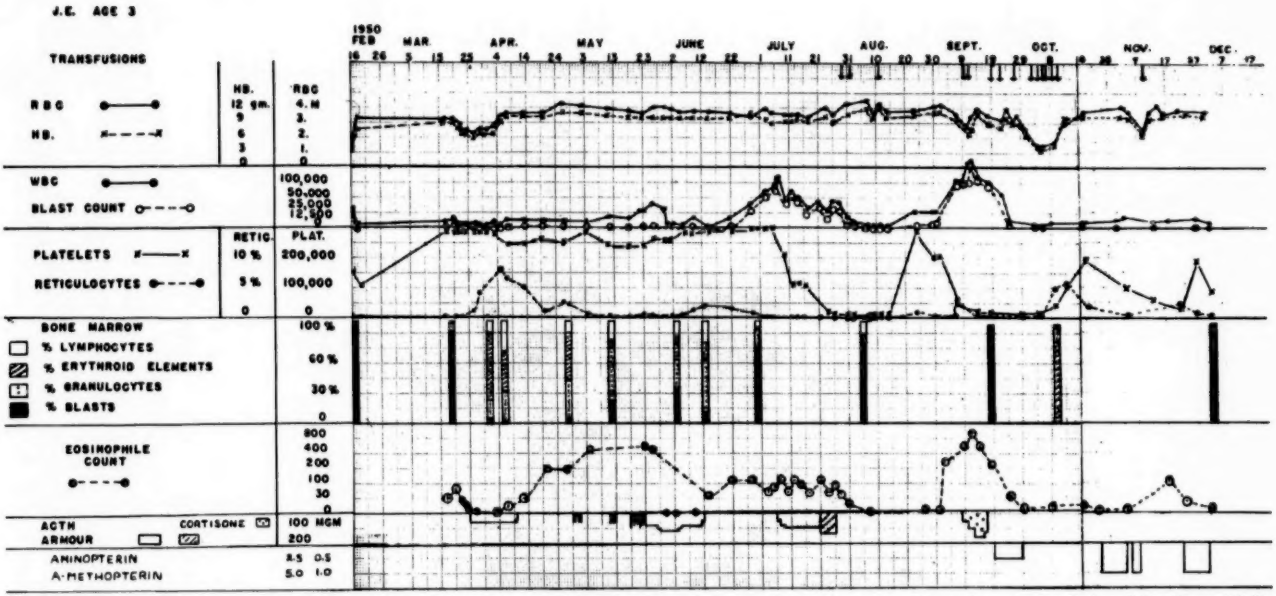


Fig. 35. Stem cell leukemia. Initial remission induced by ACTH. Duration of the remission 4 months. Second remission induced by the antifolic drugs. Total duration of life 11 months.

the average duration of the first remission was six weeks, with a range of two to 16 weeks (see Table 21). The number of children who had complete remissions to successive courses of ACTH and the duration of the remissions diminished with each course; 75 per cent responded to the first course, 52 per cent to the second course, 40 per cent to the third course, and 16 per cent to the fourth course. In anticipation of the shortness of the remission, examination of the bone marrow is indicated at monthly intervals throughout the remission, particularly if intermittent therapy is used, and even though the blood findings indicate a sustained remission.

TABLE 21. *Treatment of 37 Cases of Acute Leukemia in Children with ACTH*

REMISSION RATES OBTAINED WITH SUCCESSIVE COURSES OF ACTH

Course of ACTH	Number of Cases Treated	Number of Cases with Remissions	Remission Rate	Duration of Remission	
				Average	Range
1st	37	28	75.67%	6.5 weeks	2–16 weeks
2nd	23	12	52.50%	6.2 weeks	2–12 weeks
3rd	15	1	40.00%	4.6 weeks	2– 7 weeks
4th	6	1	16.00%	5.5 weeks	5– 6 weeks
5th	2	1	—		
6th	1	0	—		

In the patients who respond with an incomplete remission to adequate doses of ACTH or steroid administered for three weeks, we have found it unprofitable to increase the dose or to prolong therapy to the point of unfavorable signs of steroid therapy, since the remissions so obtained have not been sustained long enough to justify the risks. Therefore, if the hematological remission is incomplete or if it fails to occur at all, a chemotherapeutic regimen is instituted.

REGIMEN FOR HORMONE THERAPY. Our current regimen for hormonal therapy is as follows:

1. ACTH gel, 60 to 80 units per day, is given intramuscularly in two divided doses for children under four years of age, and 80 to 120 units for the older children. The hormone is continued for 14 to 21 days and withdrawn gradually after evidences of a marrow remission have appeared.

Steroid dosage: Cortef, 60– 80 mg. per day orally for younger children
80–120 mg. per day for older children
Meticorten, 10– 15 mg. per day in the younger children
20– 40 mg. per day in older children.

Children who receive steroid therapy are hospitalized until improvement justifies an out-patient type of care.

2. All patients are maintained on a low caloric, high protein, low salt diet with additional potassium as KCl, 0.3 to 0.6 gm. orally per day, vitamins A, D and C as multivitamin preparation. Daily physical examinations were directed toward change in the symptoms and signs relative to the leukemia status, and/or indicative of excessive steroid administration such as hypertension, water retention, emotional disturbances, glycosuria, and the like.

3. Antibiotic therapy in therapeutic doses with a broad-spectrum antibiotic is used throughout the initial hormone treatment in all children whose initial blood and marrow pattern revealed severe suppression of myeloid elements. Specific antibiotics are used as indicated by bacteriological methods.

TABLE 22. *Initial Remissions Obtained with Adrenocortical Hormones in 142 Cases of Acute Leukemia in Children*

YEAR TREATED	HORMONE	NUMBER OF CASES	REMISSIONS					
			Complete		Incomplete		Failures	
			Cases	%	Cases	%	Cases	%
1950–1	ACTH	37	28	75.67	2	5.40	7	18.91
1952–4		40	24	60.00	5	12.50	11	27.50
1955		23	14	60.87	4	17.40	5	21.73
Total	ACTH	100	66	—	11	—	23	—
1955–6	Steroid							
	Cortef	22	13	59.0	1	4.44	8	38.60
	Meticorten	15	5	33.3	2	13.33	8	53.33
	Cortisone	5	1	—	2	—	2	—
Total	Steroid	42	19		5		18	

4. Transfusions of whole blood or plasma are given as indicated by the degree of anemia and/or hemorrhagic tendency, but when possible are withheld to evaluate the response to the therapy.

5. The minimal hematological data obtained are hemoglobin, microhematocrit, reticulocyte percentage, platelet, leukocyte and differential counts.

6. Blood chemical determinations have been limited to an initial evaluation of the electrolyte pattern, nonprotein nitrogen and total proteins, albumin-globulin ratios. Subsequent chemical determinations are obtained as indicated by the chemical status.

7. Bone marrow examinations are done after the leukemic blasts have been cleared from the blood, and when a rise in the granulocyte, reticulocyte and platelet levels indicates a regeneration of myelopoiesis in the marrow. When a marrow remission is complete and the blood and

clinical status is satisfactory, no further therapy is given until evidence of a relapse develops.

Results of Hormone Therapy. Of 100 children treated with ACTH, 66 responded with a complete hematological and clinical remission, 11 with an incomplete remission, and 23 failed to respond (see Table 22). Of 42 children treated with steroids, the number of cases treated with Cortef (hydrocortisone) and Meticorten (prednisone) is large enough to allow comparisons; of 22 children treated initially with Cortef, 13, or 59 per cent, responded with a complete remission, and of 15 children treated with Meticorten, 5, or 33 per cent, showed a complete remission. A correlation between the dose levels and the type of hormone given and the remission rate is suggested by the results obtained. In the first 37 cases crystalline ACTH was administered at intervals of six or eight hours; a remission rate of 75 per cent was obtained. For the remainder of the group ACTH Gel was available and was administered at 12 to 24 hour intervals, since the long-acting preparations seemed preferable for long-continued parenteral therapy in a child vulnerable to purpuric bleeding at the sites of injection; the remission rate with ACTH Gel was 60 per cent. Cortef was more effective than Meticorten. The dose of Cortef ranged from 2 to 6 mg. per kilogram per day, and no correlation was established between the dose and the remission on a weight basis. Complete remissions were obtained with doses of 2.0 mg. per kilogram as well as with higher doses of 4 to 6 mg. However, the less favorable results with Meticorten suggest that Meticorten was given in inadequate doses or is a less potent antileukemic steroid than Cortef.

Summary. In summary, our experience with the adrenocortical hormones in the treatment of acute leukemia in children indicates that complete remissions occur in roughly two thirds of the cases treated, and an additional 10 per cent show incomplete remissions. The stem cell types respond better than the myeloblastic and monocytic types. The promptness of the palliative relief of symptoms and rapidity of the antileukemic effect on the leukemic tissues which can be attained without toxic effects in the majority of cases are the main indications for the choice of hormone therapy in the child who shows moderately advanced signs of the disease at the time the diagnosis is made. The contraindications for prolonged hormone therapy as a maintenance therapy are (1) the shortness of the duration of the remissions and (2) the hazard of increased susceptibility to infection.

The Folic Acid Antagonist Drugs

The chemotherapeutic approach to childhood leukemia dates to the early period of chemotherapy when the principle of the antimetabolite action of chemical compounds was established. The base lines were set

by the work of Quastel and Woodridge, who, in 1928, showed that a structural analogue of a moiety of a metabolic system could inhibit its biological activity. In 1948 Farber and his co-workers applied this principle in the search for a chemotherapeutic agent which might have a selective action against the leukemic cell. Of the several analogues prepared by Subbarow,[64] the most potent biological antagonists are those compounds in which the hydroxyl group in the 4 position of the

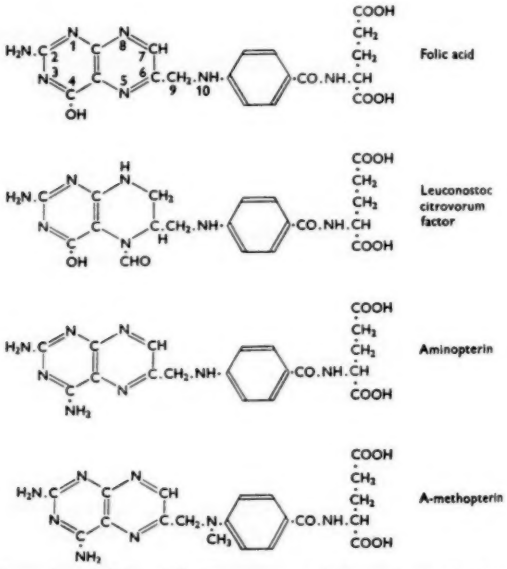


Fig. 36. Formulas of folic acid, citrovorum and the antagonists Aminopterin and Methotrexate. In all formulas the pteridine ring is on the left, the para-amino-benzolglutamic acid moiety towards the right. (From W. Jacobson. *J. Physiol.* Vol. 123.)

pteridine ring is replaced by an amino group. Aminopterin (4-amino pteroylglutamic acid) and Amethopterin (Methotrexate) (4-amino-10-methyl pteroylglutamic acid) are the analogues which have proved to be most effective in their antileukemic effect (see Fig. 36). These two drugs have stood the test of time in proving their usefulness as antileukemic agents. Furthermore, the considerable research directed specifically to the mode of action of the folic acid antagonist drugs has broadened knowledge in the field of biochemistry of folic acid in particular, and of the metabolism of nucleic acids in general. The reader is referred to the recent reviews of the extensive literature on the folic acid conjugates and antagonists by Petering,[65] Welch and Nichol[90] and

Burchenal for a more detailed discussion of this subject than can be given here.

Mode of Action of the Folic Acid Antagonists. Folic acid (FA) exists in nature in equilibrium with its derivative citrovorum factor (CF); both compounds have been isolated and synthesized. The detection of citrovorum factor was made possible by the availability of the microorganism *Leuconostoc citrovorum*, the growth of which is supported by citrovorum factor, but not by folic acid; both citrovorum factor and folic acid support the growth of *Streptococcus faecalis* and *Lactobacillus casei*. Synthetic folic acid (pteroylglutamic acid) is converted to folinic acid by an enzyme system present in liver and kidney, and this conversion is augmented by ascorbic acid. It is assumed that folic acid in tissues is converted naturally to citrovorum factor by a similar series of enzymatic reduction and formylation reactions. Citrovorum factor is considered to be the more readily utilizable of the compounds, while folic acid and its conjugates are held in reserve as provitamins (see Fig. 36).

The derivatives of folic acid play an essential role in the biosynthesis of nucleic acid. They are concerned with the transfer of single carbon units in the formation of the heterocycles which form the primary units of the polynucleotides of the nucleic acids. Greenberg[35] showed that citrovorum factor is necessary for the transfer of one carbon unit, formate or formaldehyde, to glycine amide ribotide, which is thus formulated to give the 8-carbon of the purine skeleton, and also to 5-amino 4-imidazole carboxamide ribotide to form the 2-carbon of inosinic acid (see Fig. 36).

The analogues Aminopterin and Methotrexate appear to exert their antimetabolite effect by blocking the conversion of folic acid to citrovorum factor. This effect has been demonstrated in studies with bacteria[9] and the experimental animal[62, 70] and in leukemic cells.[48] Besides inhibiting the enzymatic conversion of folic acid to citrovorum factor, there is some evidence that the antagonists alter citrovorum factor in such a way that its utilization is impaired. The more recent observations of Nichol[63] suggest that an intermediate labile factor is present in the conversion of citrovorum factor, and that this labile factor is the specific target of the antagonist effect. In studies of purine metabolism in normal and leukemic mice Skipper[79] has shown that the incorporation of formate into the purines and pyrimidines in nucleic acid is reduced in animals treated with Aminopterin, but that animals who have become resistant to the drug do not show this defect in purine metabolism.

The effect of the folic acid antagonists is reversible by citrovorum factor under certain conditions. Since citrovorum factor is a biological inhibitor for both Aminopterin and Methotrexate, it would be expected that an excess of citrovorum factor would protect against a deficiency created by the antagonist. Burchenal[12] shows that citrovorum does indeed protect the experimental animal against the effects of the analogue, and that it also counteracts the antileukemic effect in mice; Schoenbach[71] reported the protective action of Leukovorin (synthetic folic acid) against the toxic effects of Aminopterin in leukemic patients. Folic acid provides no such protection. The time factor between administration of the antagonist and the protective dose of citrovorum factor is crucial, since the effect of the antagonist can be prevented only before it combines with the enzyme system which it blocks. Goldin[34] showed

that complete protection against the lethal action of Aminopterin in mice is afforded by citrovorum factor administered one hour before, simultaneously with or one hour after the administration of the antagonist, but that no protection was obtained by administration of citrovorum factor eight hours after the antagonist. The degree of protection an animal holds against the toxic effects of Aminopterin depends upon the level of citrovorum factor in the tissues at the time of drug administration. If the tissue reserves are sufficient to permit the lethal effect on the leukemic tissue before irreversible damage is done to other susceptible cells, the total antileukemic effect, as measured by survival time, is enhanced.

The more lethal effect of the folic acid antagonist drugs on leukemic cells as compared with normal leukocytes is probably due to their higher requirement for folic acid and its conjugates; Bethell and Swendseid[5] showed that leukemic leukocytes contain fivefold the amount of citrovorum that normal leukocytes do. The morphological evidence that the growth of leukemic cells is inhibited is provided by tissue culture studies of leukemic leukocytes; Jacobsen[50] demonstrated in tissue cultures of mouse and of human leukemic leukocytes that the folic acid antagonist drugs prevent the cells from entering anaphase, thereby causing an arrest of mitosis at the metaphase of cell division; Kieler and Kieler[52] reported similar observation in tissue cultures of mouse leukemic cells.

Besides their direct effect on the leukemic cell, the folic acid antagonist drugs produce hypertrophy of the adrenal cortex[44] with loss of sudanophyllic material, which causes further lympholysis and atrophy of the lymphatic tissues. Adrenalectomy minimizes the lymphopenic effect of these drugs,[23] although the damage to the bone marrow[51] is as profound in the adrenalectomized rat as in the intact animal. During the early clinical trials with these compounds severe clinical reactions were encountered frequently, and it was suspected that the clinical and hematological remissions were related to the severity of the toxic manifestations of the drug. It is now clear that remission can be obtained without signs of toxicity, and that although the adrenal, as well as the hematopoietic, system is affected by these drugs, the beneficial effects in leukemia cannot be ascribed to a stress reaction mediated by way of the pituitary-adrenal axis.

The essential limiting factor in the usefulness of the folic acid antagonist drugs in the treatment of leukemia, aside from their toxicity, is the ability of the leukemic cell to develop resistance to the drugs. The phenomenon of resistance to the drug develops in bacteria,[46] in leukemic mouse cells[18] and in human beings, but not to the extent that the body is protected against toxicity. Burchenal and his co-workers[13] demonstrated that the resistance which develops in the leukemic cell is true cellular resistance rather than a host resistance; a strain of mouse leukemia which was originally suppressed by 4-amino pteroylglutamic acid and later was not controlled by it retained its resistance to this analogue after transfer by cell suspensions to other mice which had not been treated previously with the drug. Further, these investigators showed that resistance to one of the antifolic analogues was effective against all these analogues tested.

Although the mechanism by which the cell acquires resistance is not clear, it seems that it does not involve a change in the structure of the analogue, but that metabolic adjustments within the cell enable it to synthesize its nucleic acid by other pathways. In studies on the growth requirements of *S. faecalis*/A, an Ametopterin-resistant variant of the sensitive parent strain, Hutchison and Burchenal[47] found that this organism has a definite requirement for purines, and that it has one third the requirement of folic acid in the presence of purines; they suggest that the resistant strain is more dependent on preformed purines for the synthesis of nucleic acid than upon the synthesis *de novo* of the nitrogenous heterocycles in which folic acid plays an essential role. The cross resistance which the cell develops for the various folic acid antagonist analogues does not carry over to the drugs of the purine inhibitor series (6-mercaptopurine).

CLINICAL EFFECTS OF THE FOLIC ACID ANTAGONIST DRUGS. *Toxic Effects.* The symptoms of toxicity produced by the folic acid antagonist

drugs in animals and man are similar to those caused by a nutritional deficiency of folic acid. The more damaging effects on certain tissues which are actively concerned with mitotic division and replacement of rapidly utilized cells are apparent in the selective toxic effects on the epithelium of the mouth, gastrointestinal tract and the bone marrow. The signs of toxicity are seen first in the mouth as a stomatitis develops; shallow yellow ulcers appear in the buccal mucous membrane adjacent to Stensen's duct, which spread to the pharyngeal pillars and soft palate. These buccal lesions may be the only sign of toxicity, but, if ignored, they may be followed by more ominous manifestations in the skin and gastrointestinal tract. The cutaneous signs are heralded by a bright red erythematous rash which begins in the scalp and extends downward onto the forehead, cheeks, neck and trunk. As a rule, the stomatitis and skin rash are accompanied by symptoms of anorexia, nausea or abdominal pain and diarrhea. The severest gastrointestinal symptoms such as vomiting, mucous diarrhea and melena are accompanied by the pathological findings of extensive areas of denuded epithelium with multiple hemorrhagic ulcers in the hyperemic mucosa through the entire bowel. Alopecia occurs fairly regularly after severe signs of toxicity; thinning of the hair is common after long periods of therapy.

The hematological signs of toxicity are dependent upon the degree of damage which the drugs inflict on the various cellular constituents of the bone marrow. The erythrocytic series may show megaloblastic transformation within the marrow and macrocytosis in the peripheral blood, although more commonly there is no evidence of a maturation arrest, but rather a depletion of all the erythrocytic precursors in the marrow which results in an anemia of the aplastic type. Suppression of granulopoiesis and of megakaryocyte formation leads to leukopenia and thrombocytopenia. These manifestations of an acute aplasia in the marrow are reversible on prompt withdrawal of the drug, but after prolonged administration a chronic hypoplasia of the marrow develops.

The Folic Acid Antagonist Drug Regimen. This is as follows:

Methotrexate. The dosage is 1.25 mg. per day for children under 18 months, or older if small; 2.5 mg. per day for children aged two to eight years; 5.0 mg. per day for older children (maximum daily dose). The route of administration is oral. The dose is continued until a remission is established, and then withdrawn temporarily.

Maintenance therapy is continued, at the same dose level, and withdrawn when signs of toxicity develop. If only minimal signs of toxicity are present, i.e., stomatitis, Leucovorin may not be administered, but if further signs develop within the first 24 hours after withdrawal of the drug, it is given as Leucovorin, 3 mg. intramuscularly.

Steroids Combined with Methotrexate. Steroids are combined with Methotrexate if the marrow is hyoplastic or if the patient is acutely ill,

or in active relapse after a previous course of ACTH or steroid. The dose and preparations used have been scaled to a maintenance level:

Cortef, 10–20 mg. per day
Meticorten, 5–10 mg. per day

Reactions. Severe reactions to the folic acid antagonist drugs have occurred only rarely since the first year of our experience in handling these drugs. Several factors could contribute to the improvement in our management, the most important of which are the gains in experience in recognizing the early signs of toxicity, which indicated prompt withdrawal of the drug and administration of Leucovorin intramuscularly to counteract the effect of the antagonist received within the preceding 12 to 24 hours. In addition, we have made two changes in drug therapy. Methotrexate (Amethopterin) has been substituted for Aminopterin, and steroids in low doses have been given in combination

TABLE 23. *Results of Treatment with the Folic Acid Antagonist Drugs in Acute Leukemia in Children*

DRUG	YEAR TREATED	NUMBER OF CASES	REMISSIONS					
			Complete		Incomplete		Failures	
			Cases	%	Cases	%	Cases	%
Aminopterin	1948–50	33	15	45.44	8	24.24	10	30.30
Methotrexate	1950–6	96	28	29.06	27	28.12	41	42.70
Total		129	43	33.33	35	25.58	51	39.53

with the Methotrexate when signs of a pancytopenia occurred. The choice of Methotrexate in preference to Aminopterin was made on the basis of a wider margin of safety in the therapeutic dose, which is fivefold that of Aminopterin. The difference in the potency of the two drugs appears to permit a longer interval between the onset of toxic effect and severe signs of toxicity.

The favorable effect of steroids used in combination with the antifolic drug is related to the stimulating effect of the steroid on myelopoiesis which hastens the recovery of normal marrow elements after the antifolic drug has destroyed the blasts. We have preferred to avoid the use of steroids in high doses which produce dissolution of the lymphatic tissues simultaneously with the antileukemic effect of the antifolic compound.

RESULTS. The results of treatment with the folic acid antagonist drugs in 129 cases of acute leukemia in children are summarized in Table 23.

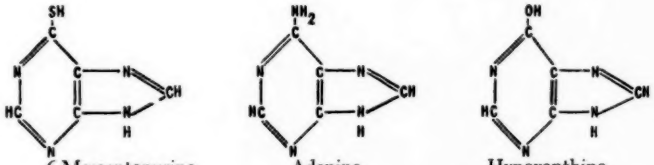
The first group of 33 children treated during 1948–9 yielded a complete remission rate of 45 per cent and an incomplete remission rate of 24 per cent; 25 of these 33 patients had received no previous antileu-

kemic drug. The group of 96 children treated after 1950 yielded a complete remission rate of 30 per cent and an incomplete remission rate of about 28 per cent; the majority of these children had been treated previously with hormones or 6-Mercaptopurine. The use of the antifolic drug earlier in the course of the disease is the most important factor in the difference in the remission rate in all probability, although the therapeutic regimens differed in other respects as well. Aminopterin without steroids was used in the first series and Methotrexate often combined with steroids in the latter group.

The toxic reaction to the drug can be avoided by careful clinical control and by the combination steroid therapy. The prolonged use of steroids during maintenance therapy with Methotrexate has the disadvantage of producing resistance to hormone therapy, which may be required later.

6-Mercaptopurine (Purinethol)

Mode of Action. The synthesis of another antimetabolite, 6-mercaptopurine, was accomplished by Hitchings and Elion[27] of the Wellcome Laboratories in 1952. It is an analogue of adenine, one of the



6-Mercaptopurine Adenine Hypoxanthine

Fig. 37. Formulas of the purines, adenine and hypoxanthine and the purine antagonist 6-mercaptopurine.

purine constituents of nucleic acid, and its physiological purine base hypoxanthine (see Fig. 37). It inhibits the growth of *Lactobacillus casei*, and this inhibitory effect is reversed by the purines adenine, guanine and hypoxanthine.[28]

The mode of action of this purine antagonist differs from that of the folic acid antagonists by interfering, not with the synthesis *de novo* of nucleic acids, but presumably, according to Brown,[10] by the incorporation of the analogue *in toto* into the nucleotide of the macromolecule in place of adenine (see Fig. 38).

Support for the concept that the analogue replaces adenine within the cell was obtained by Elion et al.;[26] by using labelled 8-C 14 and S-35 6-mercaptopurine, the radioactivity was recovered in the purine moiety of both the deoxyribonucleic acid and the ribonucleic acid of viscera of mice. In similar isotopic studies on the fate of 6-mercaptopurine in man, after an intravenous dose of the S-35 labelled analogue, Hamilton and Elion[41] also recovered the radioactivity in the deoxyribonucleic acid

of leukemia cells. However, as these investigators point out, until the actual metabolites represented by the total radioactivities are identified, proof is lacking that an actual replacement of the purine moiety by the analogue has in fact occurred.

The antitumor effect of 6-mercaptopurine was demonstrated first on the Crocher mouse sarcoma 180; Clarke et al.[15] treated mice bearing the tumor with intraperitoneal injections of the drug and observed resorption of the tumor and recovery without metastases in a significant number of animals. Later several other rodent tumors were similarly inhibited. In tissue cultures of rodent tumors and human tumors Biesle[7]

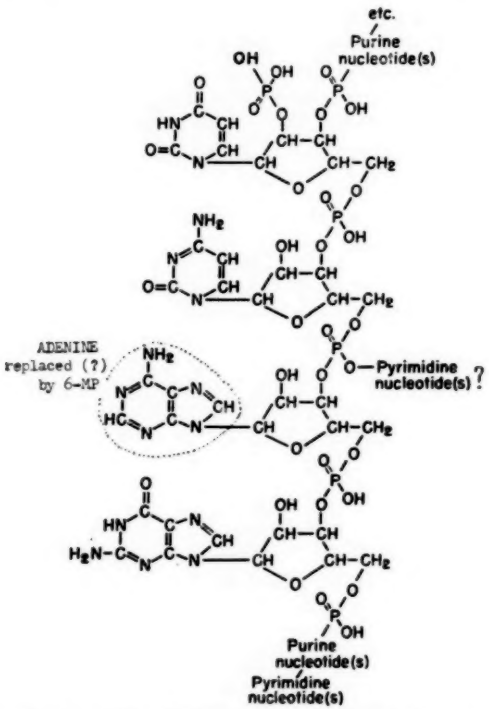


Fig. 38. Fragment of a ribose polynucleotide. (From G. B. Brown: *Ann. New York Acad. Sc.* Vol. 60.)

noted that mitosis in the malignant cells was suppressed by 6-mercaptopurine. The antileukemic effect against mouse leukemia was demonstrated by Law[55] and Skipper,[79] but in the strain L4946 no retarding effect of the drug against the leukemia could be shown.[80] In the mouse leukemias, resistance to the drug was demonstrated in the early trials, and this resistance was accompanied by a similar resistance to other purine analogues tested.[56] The inhibitory effects of 6-mercaptopurine on the growth of sarcoma 180 in mice have not been reversed by the purines adenine, guanine and hypoxanthine, or other purines tested, or by folic acid or its conjugates.[15] Adenylic acid reversed the toxic effect in mice, according to Goldin,[33] but with loss of the antileukemic effect.

The fate of 6-mercaptopurine was studied by isotopic methods in a

child with stem cell leukemia and an adult with chronic myeloid leukemia by Hamilton and Elion.[41] The results indicated that the drug is metabolized rapidly, disappearing from the plasma almost completely within 24 hours, and excreted by the kidneys mainly as a result of glomerular filtration. The half-life of the labelled isotope in the blood was 90 minutes; the plasma fraction of the blood was cleared of the isotope after two days, although small amounts were detectable in the whole blood, presumably in the cells, on the twenty-ninth day. The urinary excretion was prompt, and 60 per cent of the activity of the isotope was recovered from the urine 24 hours after administration; the chematographic studies indicated that the drug is rapidly metabolized as thioniric acid, sulfate and an unknown metabolite. Detection of radioactivity in the cerebrospinal fluid at 30 and 60 minutes after the administration of the isotope indicated that at the dose level of 6 mg. per kilogram 6-mercaptopurine passes the blood-brain barrier; however, it is not known whether the usual dose of 2.5 mg. per kilogram provides a level of the drug which is therapeutically effective.

The toxic effects of 6-mercaptopurine in the experimental animal include hypoplasia of the bone marrow, degenerative changes in the crypt cells of the intestinal epithelium, disturbance in hepatic function and/or hepatic necrosis. Toxicity studies by Philips, Clarke et al.[67] with mice and dogs determined the dose range for clinical trials, later used by Burchanel and his co-workers[14] for clinical studies. In dogs, doses of 10 mg. per kilogram intravenously for four days produced weight loss, anorexia, impaired hepatic function and leukopenia; recovery was complete four weeks after the drug was stopped.

Therapeutic Regimen. The dosage used in the first clinical trial by Burchenal et al.[14] was 2.5 mg. per kilogram per day; this dose level is still used by most of the hematologists, although some investigators prefer a higher dosage intermittently.[40]

In our center we have used 2.5 mg. per day, plus or minus 0.5 mg. per kilogram. The effect of this dosage upon the hematological picture is delayed as a rule for seven to 10 days; then, particularly if the total blast count is very high, the leukocyte count may fall sharply over a period of two to three days. A precipitous drop from a high level to 50 per cent of the initial count is an indication for withdrawal of the drug for 48 to 72 hours, since a continued fall after cessation of therapy can be anticipated, and the rapid destruction of leukemic cells leads to a rise in the blood levels of uric acid and to excessive excretion of urates, which may cause urate retention in the kidney and renal failure (see Fig. 39).

If the pretreatment level of the leukocyte count is not excessively high, the fall in the total leukocyte count is more gradual as a rule, beginning seven to 10 days after onset of therapy and continuing over a period of two to three weeks to a leukopenic level which may drop to 1500 or below before there is a reversal in the percentage of granulo-

cytes to lymphocytes in the peripheral blood, indicative of regeneration of myelopoiesis in the marrow, and recovery from the leukopenia occurs. The leukopenia is accompanied by progressive elimination of the leukemic blasts from the hematopoietic organs, and a shrinkage in the size of the peripheral lymph nodes, liver and spleen. Examination of the aspirated bone marrow is helpful in determining whether withdrawal of the drug is wise, if the leukopenia is severe; if the marrow is still heavily infiltrated with blasts, even though the peripheral leukocyte count is low, continued administration of the drug is indicated

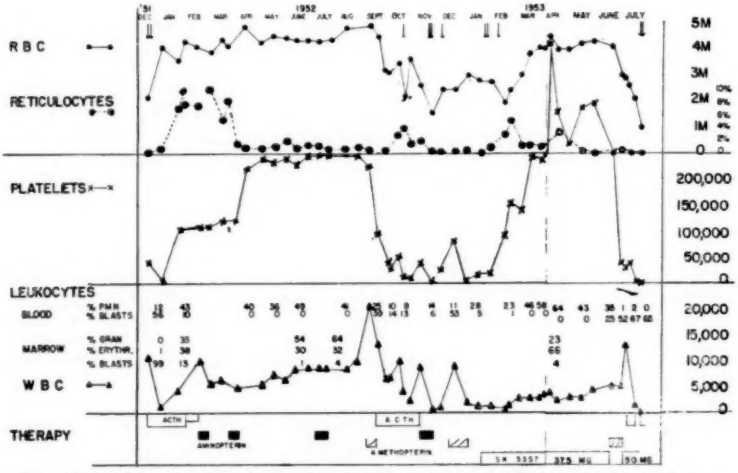


Fig. 39. Initial treatment of monoblastic leukemia with ACTH resulted in an incomplete remission. Aminopterin, 0.5 mg. daily, brought the hematological remission to completeness. On relapse neither ACTH nor the antifolic drugs were effective. 6-Mercaptopurine (SK 5357) resulted in another complete remission. The total duration of life was 21 months.

even though the count is in the range of 1000 to 1500. When it drops below this level, it has been our policy to withdraw the drug for a few days to allow for recovery before resuming the drug. In our experience the most common sign of toxicity of 6-mercaptopurine has been the leukopenia, which has necessitated withdrawal of the drug. Other signs of toxicity have been minimal; stomatitis has been rare, and gastrointestinal symptoms other than anorexia have not been encountered without signs of marrow aplasia. In a few children who have taken the drug for several weeks, anorexia, thinning of the hair and a vague abdominal cramp have developed and disappeared on withdrawal of the drug.

Results of Treatment. In 74 children treated with the drug 22, or 29.73 per cent of the cases, yielded complete marrow and clinical remissions, and 24, or 32.43 per cent of the cases, gave incomplete

remissions, and 28, or 37.83 per cent, were failures. Only eight of these children received 6-mercaptopurine as the first antileukemic treatment; in 62 children it was used after a course of steroid and/or one of the antifolic drugs. The lower remission rate with 6-mercaptopurine attained in our center is undoubtedly due to the fact that in the past it was used at a later stage in the disease than in the centers where it is used as the initial treatment or immediately after a course of hormone therapy.

In our current series of cases, admitted during 1956, mercaptopurine, alone or in combination with Azaserine, has been used as the first chemotherapeutic regimen. The more severely ill patients receive a

TABLE 24. *Results of Treatment with 6-Mercaptopurine in Acute Leukemia in Children*

YEAR TREATED	NUMBER OF CASES	REMISSIONS					
		Complete		Incomplete		Failures	
		Cases	%	Cases	%	Cases	%
1953–4.....	32	10	31.25	13	40.62	9	28.12
1955.....	42	12	28.57	11	26.19	19	45.23
Total.....	74	22	29.73	24	32.43	28	37.83

course of hormone therapy prior to the drug regimen, and chemotherapy is instituted only after relapse has occurred or when the remission to hormones is incomplete. In the less severely ill cases the chemotherapy regimen is instituted immediately.

DURATION OF LIFE

An evaluation of the current methods of treatment of leukemia is difficult to measure by any other standard than the prolongation of life. The clinical data[11, 40, 68] accumulated in the several pediatric centers where therapeutic programs have been conducted within the past few years indicate that the life span of children who respond to the chemotherapeutic and hormonal regimens is prolonged significantly as compared with the duration of life in cases treated prior to the advent of the specific antileukemic drugs.

In Table 25 a comparison is given of the percentage of the children who have survived longer than six months under different types of treatment, in programs conducted by the writer. The most significant improvement in the prolongation of life occurs in the children who have received more than one antileukemic treatment; in 51 cases treated with steroids followed by the folic acid antagonist drugs and in 16 cases treated with steroids and 6-mercaptopurine duration of life was

doubled as compared with that of the control series of 55 cases treated with transfusions only. In 54 cases treated with steroids, Methotrexate and later 6-mercaptopurine, the duration of life was trebled. More impressive than the improvement in the average duration of life is the extension of life beyond a year; 35 per cent of the children treated in sequence with the three antileukemic regimens lived beyond a year, 16 per cent beyond 15 months, and 3.7 per cent beyond 18 months; no patient in the deceased group lived as long as two years. In our current series of 35 cases, six children have been under treatment for more than 21 months; in two cases the remission is complete, in two

TABLE 25. *Duration of Life Compared with Therapy in 240 Fatal Cases of Leukemia in Children*

DATES TREATED	THERAPY	NUMBER OF CASES	AVERAGE DURATION OF LIFE	MONTHS OF SURVIVAL AFTER ONSET						
				Less than 6	6–8	9–12	12–14	15–17	18–21	21+
				Percentage of Children						
1936–41	Transfusion only	55	16 weeks	81.8	12.7	5.4	—	—	—	—
1946–8	P32	26	23 weeks	69.3	15.3	7.3	3.8	—	—	—
1948–50	FAA alone	25	25 weeks	48.0	40.0	12.0	—	—	—	—
1950–6	Hormones alone	21	14.6 weeks	85.7	14.3	—	—	—	—	—
1950–6	Steroid and FAA	51	32.3 weeks	31.0	33.0	16.8	9.8	1.4	5.8	—
1953–6	Steroid and 6-MP	16	31.1 weeks	37.5	18.5	25.0	6.2	6.2	6.2	—
1953–6	Steroids, FAA 6-MP in sequence	54	43 weeks	9.2	33.3	22.3	18.5	12.9	3.7	—

children the clinical remission is good, although hematological remission is now incomplete, and two children have recently had neurological complications.

The toll of life in the first six months of the disease is indicated clearly by the high percentage of patients whose survival time is shorter than six months after the onset. After 1950, when more than one drug became available, the children who fail to respond to the initial therapy now fall into this group; in the main, this is the group of children whose illness is most severe in the early stages of the disease. The failure of the adrenocortical hormone therapy to improve the duration of life is a poor indicator of the benefits obtained from hormone therapy in the critically ill child. The high remission rate provided by the initial treatment with hormones reduces the mortality rate in the first six months of the disease, and thereby improves the duration of life obtained with the antagonist drugs.

The criterion of the life span neglects the issue most imperative to the clinician, namely, the total benefit which the modern regimens of therapy confer upon the patient. In this regard the clinician necessarily must accept some of the same frustrations which beset the parents of his charge. It is still true that approximately 25 per cent of the children expire within the first six months of treatment, and that a small fraction live beyond two years. As the disease proceeds, the clinician shares the emotional stress of the family as the inevitable signs of relapse develop, when resistance to the current therapy occurs. Perhaps the most disturbing of all is the increasing percentage of neurological complications which develop in children who have remained in hematological remission. Until further knowledge in the pathogenesis of the disease becomes available, these are problems which the clinician must anticipate.

At present it can be said that the child benefits from the clinical improvement which accompanies the hematological remissions induced by hormones or chemotherapy; weeks and months of a near-to-normal life are provided in a significant percentage of cases treated. In spite of the limitations of the present methods of treatment, the results justify the active effort not only to improve the results achieved with the current methods, but also to expand the research efforts in the development of new antileukemic drugs and steroids. Until these new advances occur, improvement in the results obtained with our present methods depends largely upon the ability to detect the disease in its early stages, to control its progress as rapidly as possible with effective antileukemic therapy and to treat the complications inherent in the disease, as well as secondary infections, as vigorously as our knowledge in general pediatrics permits.

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HEMORRHAGIC DISORDERS

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The maintenance of normal hemostasis is a complex process involving the blood vessels, the blood platelets and a group of circulating plasma proteins which enters into the coagulation cycle. Though the immediate response to trauma probably depends, to a large extent, on the structural and functional integrity of the small arterioles and capillaries, the ultimate and final control of hemorrhage requires the formation of a firm clot. The extraordinary ability of the blood to remain entirely fluid while in the circulation and yet clot promptly upon being shed has intrigued physicians through the ages, and though much has been accomplished in recent years to unravel this beautifully complex mechanism, many questions still remain to be answered. The recent advances in coagulation physiology have led to much progress in the recognition, diagnosis and treatment of the patient with an abnormal tendency to bleed and thus deserve the attention of the clinician. Since the vascular and platelet abnormalities are covered elsewhere in this volume, the present discussion will be concerned with the plasma coagulation factors and the disorders resulting in and from their deficiency.

THE NORMAL COAGULATION MECHANISM

The actual formation of the clot results from the interaction of the platelets, calcium and at least seven, and probably more, circulating plasma proteins. The end point of this highly complicated process is

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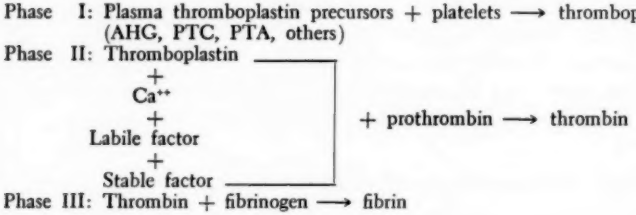
the conversion of soluble fibrinogen to the insoluble network of fibrin strands which forms the clot itself. The coagulation cycle is conveniently divided into three phases: In the *first phase* three proteins, antihemophilic globulin (AHG), plasma thromboplastin component (PTC) and plasma thromboplastin antecedent (PTA) react with the platelets to form thromboplastin. Though antihemophilic globulin, plasma thromboplastin component and plasma thromboplastin antecedent constitute the plasma thromboplastin precursors which have received most study to date, recently published experiments indicate the existence of several other factors required for formation of thromboplastin. In the *second phase* thromboplastin reacts with calcium and prothrombin to yield thrombin. The conversion of prothrombin to thrombin requires, however, two accessory factors, (1) labile factor (also called Factor V, Ac-globulin and pro-accelerin) and (2) stable factor (also called Factor VII, serum prothrombin conversion accelerator and pro-convertin). Prothrombin, stable factor and labile factor are frequently called the prothrombin complex. In the *third phase* of coagulation, thrombin reacts with fibrinogen to form fibrin.

The coagulation mechanism proceeds in two main sequences, the first being remarkably slow, the second extremely rapid. The keystone of this sequence appears to be thrombin. Thus with the formation of the first trace of thrombin, accelerators are activated which influence all three phases of the coagulation cycle.

Finally, in order to maintain the balance between fluidity and coagulability of the blood, natural anticoagulants exist which are active in all phases of the coagulation mechanism.

Here is a schematic representation of the coagulation mechanism.



CLASSIFICATION

Hemorrhagic diseases due to a deficiency of plasma coagulation factors may be classified as follows:

I. Deficiency of factors required for thromboplastin formation (the hemophilias)
  A. Antihemophilic globulin (AHG) deficiency
    1. Classic hemophilia (hemophilia A)
    2. Mild hemophilia
    3. Vascular hemophilia

B. Plasma thromboplastin component (PTC, Christmas factor) deficiency
   1. Hemophilia B
C. Plasma thromboplastin antecedent (PTA) deficiency
   1. Hemophilia C

II. Deficiency of factors of prothrombin complex
   A. Prothrombin deficiency
   B. Labile factor deficiency
   C. Stable factor deficiency

III. Deficiency of fibrinogen
   A. Afibrinogenemia
   B. Hypofibrinogenemia
   C. Excessive fibrinolysis

THE HEMOPHILIAS

Until 1952 the term "hemophilia" was reserved for a well defined, severe hemorrhagic disease which occurred in males and was transmitted by females. The disease was generally considered to result from congenital deficiency of a particular protein (antihemophilic factor), the lack of which resulted in a marked prolongation of the whole blood clotting time. In recent years, however, it has become evident that deficiencies of other factors, distinct and different from antihemophilic globulin, may produce all the clinical and laboratory manifestations of hemophilia. At present five factors in addition to antihemophilic globulin have been implicated in disease states satisfying the general criteria for hemophilia. All these factors are required for normal formation of thromboplastin. It has also been established that all grades of deficiency of the various factors may exist, with the clinical and laboratory aberrations proportional to the severity of the deficiency. These facts have made it necessary to define hemophilia in terms of the specific factor lacking. It has also become necessary to establish laboratory tests capable of detecting the mild to moderate deficiencies.

CLASSIC HEMOPHILIA (HEMOPHILIA A)

Classic hemophilia is a severe congenital disorder transmitted as a sex-linked mendelian recessive trait, i.e., carried by the female and manifested by the male. The disease results from virtual absence of antihemophilic globulin in the affected males, a state which is constant and permanent. The carrier female, on the other hand, has been found, in most instances, to have normal antihemophilic globulin levels and is usually asymptomatic. The characteristics of the inheritance are such that half of the sons of a carrier female will have the disease, while half of the daughters will be carriers. The sons of an affected male will be normal, while the daughters will be carriers.

Clinical Manifestations

Classic hemophilia is characterized by recurrent episodes of bleed-

ing which may involve any area of the body. The hemorrhages may occur spontaneously or follow slight trauma. The disease is often first indicated by the occurrence of severe bleeding following circumcision, but surprisingly enough, many hemophiliacs are apparently able to tolerate circumcision in the neonatal period with minimal difficulty. Evidence of the abnormal tendency to bleed usually becomes pronounced with the onset of walking. Hemorrhages may occur into skin, muscles, solid viscera, gastrointestinal tract, peritoneal cavity, central nervous system, and from mucous membranes. Particular difficulty in the young infant may occur after injury to lips, tongue, frenulum and gums. The characteristic manifestation of classical hemophilia is bleeding into joint cavities, ankles, knees and elbows being most frequently affected. Acute hemarthroses produce severe pain, swelling, heat, tenderness and limitation of motion, while repeated joint hemorrhages may produce extensive damage to synovial membranes, articular surfaces, epiphysial plates and metaphyses. Inflammation, degeneration and fibrosis may lead to ankylosis and severe crippling.

Laboratory Findings

In all types and grades of hemophilia the platelet count, bleeding time, clot retraction, prothrombin time and fibrinogen concentrations are normal. In severe grades of hemophilia marked prolongation of whole blood clotting time is characteristic. In milder types, however, the clotting time may be only slightly prolonged or even normal. It is important to realize that as little as 1 per cent antihemophilic globulin is sufficient to produce a normal whole blood clotting time.

A more sensitive test is the *prothrombin consumption test*, which measures the residual prothrombin in serum after clotting has occurred. Normally over 80 per cent of the available prothrombin in the test tube is consumed during clotting. In abnormalities in the first phase of coagulation inadequate amounts of thromboplastin are formed to convert the prothrombin to thrombin, and therefore abnormal amounts of residual prothrombin are found in the serum. This test, then, detects any first-phase deficiency and is not specific for the individual factors. In classic hemophilia marked impairment of prothrombin consumption is found. In milder degrees of deficiency, however, even this test may be normal, since 3 to 5 per cent antihemophilic globulin is sufficient to yield normal prothrombin consumption.

The most sensitive test available today is the *thromboplastin generation test*. In this test barium sulfate-adsorbed plasma (which contains antihemophilic globulin and plasma thromboplastin antecedent), serum (which contains plasma thromboplastin component and plasma thromboplastin antecedent), platelets and calcium are incubated together. It will be noted that the mixture contains all the factors neces-

sary for thromboplastin formation. At regular intervals aliquots of the incubation mixture are added to normal plasma; the induced speed of clotting is a measure of the thromboplastin formed. With this test levels of antihemophilic globulin below 15 to 20 per cent of normal will be detected. This is significant, since abnormal bleeding does not usually occur at higher levels. With specific modifications the thromboplastin generation test may be used to differentiate deficiencies of antihemophilic globulin, plasma thromboplastin component and plasma thromboplastin antecedent.

Treatment

The treatment of acute hemorrhagic episodes depends upon supplying to the patient an amount of antihemophilic globulin which will temporarily correct his coagulation abnormality. Recent studies have demonstrated that correction of various laboratory tests is not synonymous with correcting the coagulation mechanism in the patient. It has long been known that transfusion of small amounts of blood or plasma will effectively reduce the whole blood clotting time in hemophilia to normal. In vitro it may be demonstrated that less than 1 per cent of antihemophilic globulin will yield a normal whole blood clotting time. In these situations, however, more sensitive tests reveal persistence of marked impairment of the coagulation mechanism, a fact which may often be confirmed clinically as the patient continues to bleed despite a normal clotting time.

The prothrombin consumption test is a more sensitive index of control, but here, too, the results may be deceptive, since as little as 3 to 5 per cent antihemophilic globulin in the patient may produce normality of the prothrombin consumption test even though the patient's coagulation efficiency is still markedly impaired. Figure 40 demonstrates that between 10 and 20 per cent of normal plasma in hemophilic plasma is required to correct the most sensitive test of hemostatic efficiency, the thromboplastin generation test. If one considers the average plasma volume of the patient to be 50 ml. per kilogram of body weight, and the antihemophilic globulin concentration of the donor plasma to be 100 per cent, then a dose of plasma of 10 ml. per kilogram, will raise the antihemophilic globulin level in the recipient from zero to 20 per cent. The frequency with which plasma must be administered depends upon the longevity of antihemophilic globulin in the circulation. Results in our laboratory have confirmed the findings of Brinkhous et al.[9] that transfused antihemophilic globulin disappears in a logarithmic manner, with a half-life of four to six hours; i.e., 50 per cent of the initial level will be present after four hours, 25 per cent after eight hours, 12.5 per cent after 12 hours, etc. Thus if one were to raise

the antihemophilic globulin level to 20 per cent by an amount of plasma equal to 10 ml. per kilogram, the circulating antihemophilic globulin level in the recipient would be 10 per cent in four hours, 5 per cent in eight hours, 2.5 per cent in 12 hours.

The results of a transfusion of the above dose of plasma to a hemophiliac are shown in Figure 41. By direct assay it was found that a level of 17 per cent antihemophilic globulin was achieved. At this level all

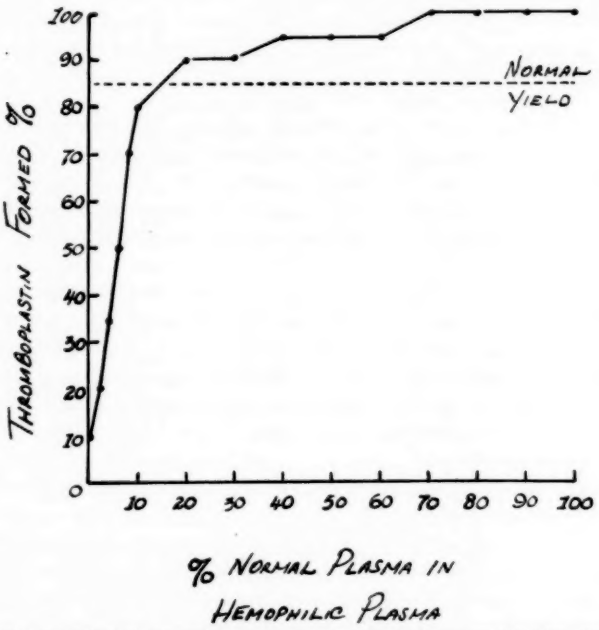


Fig. 40. The effect of increasing amounts of normal plasma on the thromboplastin generation test in severe hemophilia. Normal thromboplastin formation occurs when normal plasma constitutes between 10 and 20 per cent of the mixture.

coagulation tests were normal. Six hours after transfusion the antihemophilic globulin level was 7 per cent. At the latter point the clotting time and prothrombin consumption test were still normal, but the thromboplastin generation test disclosed severe impairment in the coagulation mechanism. The two former tests did not begin to become abnormal until 20 hours had elapsed despite the fact that only trace amounts of antihemophilic globulin could be detected in the circulation. In order to maintain a constant level of antihemophilic globulin between 10 and 20 per cent it is necessary to administer plasma in a dose of 10 ml. per kilogram initially and 5 ml. every four hours (Fig.

42). However, *in the presence of active bleeding and extensive tissue damage the rate of loss may be even more rapid than described above.*

An attempt to maintain steady and complete control of the hemostatic mechanism is necessary in severe hemorrhage, in preparing a patient for any necessary surgery, and in instances of bleeding into dangerous areas such as the tissues of the neck, floor of the mouth and

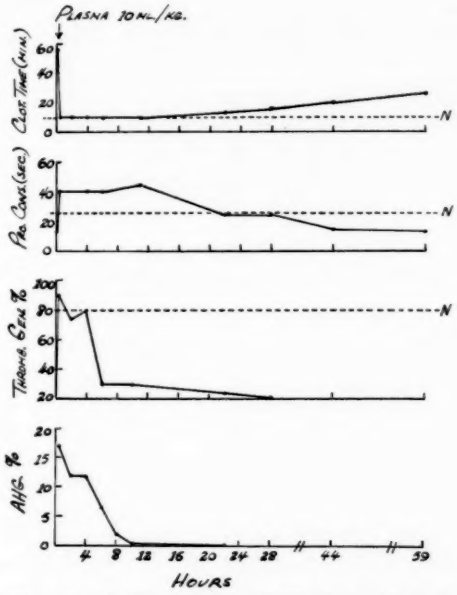


Fig. 41. The effect of a single transfusion of 10 ml. per kilogram of fresh plasma to a patient with severe hemophilia. The relation of the circulating antihemophilic globulin levels to clotting time, prothrombin consumption and thromboplastin generation is shown.

central nervous system. In less severe types of bleeding such large and frequent transfusions do not appear necessary. Thus, for example, hemarthroses may usually be treated with 10 ml. per kilogram of plasma every 24 hours for two to four days.

Antihemophilic globulin is quite labile on storage under blood bank conditions, and as much as 50 per cent of the factor may disappear in 24 hours. Therefore only fresh blood or plasma should be used. Freezing plasma within a few hours after it has been drawn maintains high levels of antihemophilic globulin for long periods of time, and many blood banks currently stock supplies of "fresh-frozen" plasma. Lyophilization of fresh plasma, when properly carried out, also seems capa-

ble of maintaining stability of antihemophilic globulin. In one commercial preparation* that we have tested the antihemophilic globulin levels averaged 70 per cent of those in fresh normal plasma and have been found stable on storage. The lyophilized material is reconstituted with diluent just before use. Fresh whole blood is usually reserved for situations in which excessive blood loss has occurred. Cohn's fraction I of plasma has not been more effective than fresh whole plasma and

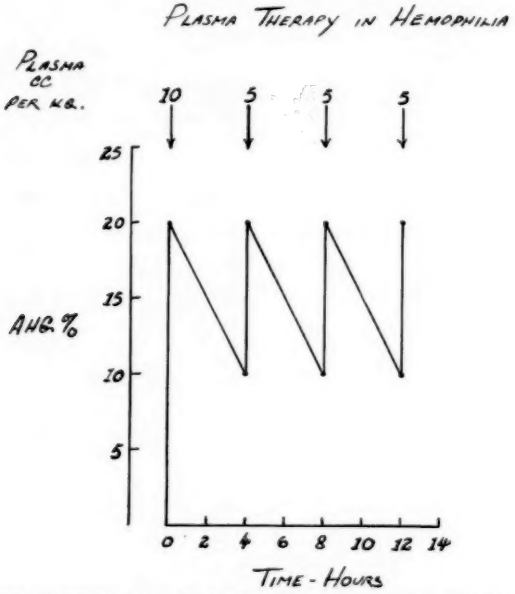


Fig. 42. Theoretical model of the transfusion regimen designed to maintain steady and complete control of hemostasis in severe hemophilia.

carries a much greater risk of hepatitis. After numerous transfusions of blood, plasma or plasma fractions some hemophiliacs develop antibodies against antihemophilic globulin and become refractory to therapy. This dangerous complication, fortunately, is rare.

Local measures are often beneficial, but most frequently constitute an addition to, rather than a substitute for, transfusion therapy. Ice packs are helpful in treatment of hemarthroses and intramuscular hemorrhage. For open wounds absorbable packs, such as Gelfoam, which have been soaked in a solution of topical thrombin and epinephrine, may be applied with gentle pressure. Suturing should be avoided

* Antihemophilic Plasma (Hyland Laboratories, Los Angeles, Calif.).

when possible, since excessive tension may lead to devitalization of the tissue, a factor which promotes further bleeding. Cauterization is always contraindicated.

Cooperation with the orthopedist is essential in order to conserve maximum function and prevent deformities. It is our opinion that hemarthroses should be treated promptly and adequately (three to four days) with plasma. After cessation of bleeding properly prescribed exercises should be undertaken in order to restore a full range of motion as soon as possible. Recent experience in our clinic has indicated that the use of prednisone (20 mg. per day) for one week after control of the joint bleeding by plasma aids in reducing spasm and periarticular inflammation and significantly accelerates return of function.

Psychological considerations require constant attention and counseling in order to prevent emotional invalidism and to prepare the child for useful adulthood.

MILD HEMOPHILIA

Whereas in classic hemophilia antihemophilic globulin levels range from zero to trace amounts, milder forms of the disease with levels ranging from 5 to 20 per cent are known to occur. Mild hemophilia appears as a familial characteristic and is postulated to be an allelic form of the disease. The affected patients give only slight evidence of a spontaneous tendency to bleed, and hemarthroses are rather uncommon. Severe hemorrhage may occur, however, after trauma or surgical procedures such as tonsillectomy or dental extractions. Whole blood clotting times are usually normal, and prothrombin consumption and thromboplastin generation tests are required to establish the diagnosis.

VASCULAR HEMOPHILIA

This disorder occurs in both males and females and is characterized by a combination of moderate to severe antihemophilic globulin deficiency plus a vascular abnormality. The latter consists of coiled, tortuous capillaries and venules which do not constrict normally on trauma. The outstanding laboratory finding is a prolonged *bleeding time;* whole blood clotting time, prothrombin time, platelet count and clot retraction are normal, but the coagulation abnormality and antihemophilic globulin deficiency may be demonstrated by prothrombin consumption and thromboplastin generation tests. Symptoms are similar to those of mild hemophilia with the exception that severe nosebleeds are often the predominant manifestation. The disease appears to be transmitted as a mendelian dominant with variable penetrance. Patients with vascu-

lar hemophilia were previously included in the classification of "pseudohemophilia." Administration of fresh plasma corrects the coagulation disturbance, but produces only transient improvement in bleeding time. Because of the associated vascular defect, patients with vascular hemophilia often pose a greater problem in management than those with the severest forms of classic hemophilia.

PLASMA THROMBOPLASTIN COMPONENT DEFICIENCY (HEMOPHILIA B, CHRISTMAS DISEASE)

This disorder is transmitted as a sex-linked recessive and comprises about 15 per cent of all hemophilias. In the severe form the clinical manifestations are indistinguishable from those of classic hemophilia. The results of routine laboratory procedures are also identical, and special tests are required for identification of the specific deficiency. Separation of the two disorders is of practical importance since plasma thromboplastin component is stable on storage, thus permitting treatment of hemorrhage with stored blood as plasma. The longevity of plasma thromboplastin component in the circulation is appreciably greater than that of antihemophilic globulin, and transfusions are usually needed only at 48 to 72 hour intervals. As with antihemophilic globulin deficiency, mild forms of plasma thromboplastin component deficiency, with comparable symptomatology, have been described.

PLASMA THROMBOPLASTIN ANTECEDENT DEFICIENCY (HEMOPHILIA C)

This third form of hemophilia differs from classic hemophilia and plasma thromboplastin component deficiency in that it affects males and females and appears to be transmitted as a mendelian dominant. The true incidence of this disorder is unknown, but recent experience indicates that it may exceed antihemophilic globulin and plasma thromboplastin component deficiencies in frequency. Plasma thromboplastin antecedent deficiency most often exists as a mild to moderate disorder which may escape detection for long periods of time because of the paucity of symptoms of spontaneous bleeding. As with mild antihemophilic globulin deficiency, the patients often present with excessive bleeding following minor trauma or such surgical procedures as tonsillectomy or dental extraction. Easy bruising or occasionally spontaneous epistaxis may call attention to the defect. Hemarthroses are rare. In most instances the whole blood clotting time is normal or only minimally prolonged. An abnormal prothrombin consumption may indicate the abnormality in the first phase of coagulation, but even the test may be normal in mild cases. The thromboplastin generation test, however, will usually detect the abnormality. Plasma thromboplastin

antecedent is stable, and stored blood or plasma given every 24 to 48 hours may be used to treat hemorrhagic episodes or to prevent excessive bleeding should surgical procedures be necessary.

DIFFERENTIATION OF THE HEMOPHILIAS

Though such tests as clotting time, prothrombin consumption and thromboplastin generation indicate deficiencies of plasma thromboplastin precursors, they do not identify the specific factor involved. Differentiation among the various hemophilias may be done with relative ease by testing for mutual correction in mixtures of blood or plasma from the test patient and from subjects with known defects. For example, blood or plasma from a patient with antihemophilic globulin deficiency will correct the abnormal coagulation tests of patients with

TABLE 26. *Distribution of Plasma Thromboplastin Precursors in Normal Plasma, Normal Serum and Barium Sulfate-Absorbed Plasma*

REAGENT	PLASMA THROMBOPLASTIN PRECURSORS PRESENT
Normal plasma	AHG, PTC, PTA
Normal serum	PTC, PTA
$BaSO_4$-adsorbed plasma	AHG, PTA

TABLE 27. *Differentiation of the Hemophilias*

REAGENT	TYPE OF DEFICIENCY		
	AHG	PTC	PTA
Normal plasma	Corrects	Corrects	Corrects
Normal serum	No correction	Corrects	Corrects
$BaSO_4$-adsorbed plasma	Corrects	No correction	Corrects

plasma thromboplastin component and plasma thromboplastin antecedent deficiency, but not of another classic hemophilic. In reverse the test patient's abnormalities will be corrected by plasma thromboplastin component- and plasma thromboplastin antecedent-deficient plasma, but not by antihemophilic globulin-deficient plasma. In the absence of a panel of plasmas with known deficiencies, identification may be made by testing the corrective effects of normal serum and normal barium sulfate-adsorbed plasma. Normal serum contains plasma thromboplastin component and plasma thromboplastin antecedent; barium sulfate-adsorbed plasma contains antihemophilic globulin and plasma thromboplastin antecedent. Thus a patient with antihemophilic globulin deficiency will be corrected by the adsorbed plasma, but not by serum; a patient with plasma thromboplastin component deficiency

will be corrected by normal serum, but not by adsorbed plasma; plasma thromboplastin antecedent deficiency will be corrected by both reagents. The plasma thromboplastin precursors found in whole plasma, adsorbed plasma and serum are summarized in Table 26. The differential patterns of correction using these reagents are summarized in Table 27. The correction experiments are usually tested on the patients' clotting time or prothrombin consumption tests. When the only normal test is the thromboplastin generation test, modifications may be made so that it, too, provides differential diagnosis. As described earlier, this test is carried out by incubating mixtures of *patient's* adsorbed plasma, *patient's* serum, platelets and calcium. If *normal* absorbed plasma and

TABLE 28. *Differentiation of the Hemophilias with the Thromboplastin Generation Test*

SOURCE OF TEST REAGENTS			RESULTS OF TGT IN VARIOUS HEMOPHILIAS		
$BaSO_4$ Plasma	Serum	Platelets	AHG	PTC	PTA
Patient	Patient	Normal	Abnormal	Abnormal	Abnormal
Patient	Normal	Normal	Abnormal	Normal	Normal
Normal	Patient	Normal	Normal	Abnormal	Normal

normal serum are individually substituted for the patient's reagents, differential patterns emerge which are summarized in Table 28.

OTHER THROMBOPLASTIN PRECURSOR DEFICIENCIES

In addition to antihemophilic globulin, plasma thromboplastin component and plasma thromboplastin antecedent, three other factors have been implicated in the first phase of coagulation. These have been designated PTF-D, factor X and Hageman factor. The former two appear to have many clinical and laboratory similarities to plasma thromboplastin antecedent deficiency. Hageman factor deficiency is particularly interesting in that the affected patients demonstrate only minimal evidence of a hemorrhagic tendency, even after surgery, despite marked abnormalities on laboratory testing.

DEFICIENCIES IN THE PROTHROMBIN COMPLEX

The prothrombin complex consists of prothrombin plus the labile and stable factors. Given adequate formation of thromboplastin, normal conversion of prothrombin to thrombin requires participation of all three factors in the complex. Prothrombin and stable factor are synthesized in the liver and require vitamin K for normal formation. Thus deficiencies of prothrombin and stable factor, usually combined, may

occur with parenchymal liver disease, dietary lack of vitamin K and with various disorders leading to impaired absorption of the fat-soluble natural vitamin (diarrhea, steatorrhea, celiac disease, gastrointestinal malformation or resection). Combined prothrombin-stable factor deficiencies are induced by administration of the coumarin group of anticoagulants. Labile factor, while also synthesized in the liver, apparently does not require vitamin K. Acquired labile factor deficiency may occur in parenchymal liver disease, malignancies, sepsis, erythroblastosis fetalis and after surgery.

Congenital deficiency of each of the three factors of the prothrombin complex also occurs. Present evidence indicates that congenital stable factor deficiency is transmitted as a mendelian dominant, while congenital labile factor deficiency is transmitted as an incomplete dominant; i.e., both parents are heterozygous, while the affected child is homozygous. The exact mode of inheritance of congenital prothrombin deficiency has not been established.

The newborn infant is born with a moderate deficiency of both prothrombin and stable factor. During the first 48 to 72 hours of life the levels of both factors fall sharply. A spontaneous rise then follows, with birth levels regained after seven to 10 days. Throughout this sequence the deficiency of stable factor is greater in degree than that of prothrombin. In premature infants the levels of both factors, both initially and during the postnatal days, are significantly lower than in the full term babies. In approximately one of 200 newborns the prothrombic activity of the plasma reaches such low levels that spontaneous hemorrhage may occur and result in the condition of "hemorrhagic disease of the newborn." The combined prothrombin-stable factor deficiency characteristic of the newborn period is thought to result from lack of vitamin K from the mother, immaturity of the liver and absence of the normal intestinal bacterial flora which synthesizes vitamin K in later days. Administration of 2 to 4 mg. of vitamin K on the day of birth is usually sufficient to prevent the neonatal fall in prothrombic activity. Excessive amounts of vitamin K should be avoided, since increased bilirubinemia and kernicterus have followed administration of 10 to 50 mg. to newborn infants.

Patients with significant deficiencies in the prothrombin complex demonstrate a bleeding tendency characterized by cutaneous hemorrhage, epistaxis, hematuria and melena. In mild deficiencies bleeding may become evident only after trauma or surgery.

Disturbance in the prothrombin complex appears to have an associated vascular component, and a prolonged bleeding time and positive tourniquet test may be found. The whole blood clotting time is prolonged only in severe deficiencies. The specific test for abnormality in

the prothrombin complex is prolongation of the one stage of prothrombin time. However, *this test does not determine the individual factor responsible for the abnormality*, and further tests must be done to identify whether prothrombin, stable factor or labile factor, singly or in combination, are involved. One method for differentiation utilizes the same "normal reagents" (serum and barium sulfate-adsorbed plasma) as used for the hemophilias. In this situation, however, the factors of the second phase of coagulation are evaluated, and the prothrombin time is used as the test to be corrected. Table 29 lists the distribution of the factors of the prothrombin complex in plasma, serum and adsorbed plasma. The ability of these reagents to correct the ab-

TABLE 29. *Distribution of Factors of Prothrombin Complex in Normal Plasma, Serum and Barium Sulfate-Absorbed Plasma*

REAGENT	FACTORS OF PROTHROMBIN COMPLEX PRESENT
Normal plasma	Prothrombin, labile factor, stable factor
Serum	Stable factor
$BaSO_4$ plasma	Labile factor

TABLE 30. *Differentiation of Prothrombin Complex Deficiencies*

REAGENT	TYPE OF DEFICIENCY CAUSING ABNORMAL PROTHROMBIN TIME		
	Prothrombin	Stable Factor	Labile Factor
Normal plasma	Corrects	Corrects	Corrects
Serum	No correction	Corrects	No correction
$BaSO_4$ plasma	No correction	No correction	Corrects

normal prothrombin time of patients with various disturbances in the prothrombin complex is shown in Table 30. It will be noted that patterns emerge which permit identification of single specific deficiencies. With combined deficiencies, however, more complicated tests are required.

Administration of vitamin K is of no value in congenital deficiencies of factors of the prothrombin complex and of slight to no value in deficiencies secondary to parenchymal liver disease. Vitamin K will produce improvement in prothrombic activity in deficiencies resulting from dietary lack or failure of absorption. With biliary obstruction or steatorrhea the vitamin must be given parenterally or, if orally, in a water-soluble form. Prothrombin and stable factor are stable on storage, and thus banked blood or plasma may be used in transfusion therapy. Labile factor, on the other hand, deteriorates rapidly on storage, and fresh blood or plasma must be used in therapy of this deficiency. The general principles of transfusion therapy described for treatment of the various hemophilias apply here.

DEFICIENCIES IN FIBRINOGEN

Congenital afibrinogenemia, in which there is almost complete absence of the protein, affects both males and females and appears to be transmitted as a partial dominant. A high incidence of consanguinity has been noted in parents of affected children. In congenital afibrinogenemia the blood is incoagulable, and yet, interestingly enough, the hemorrhagic manifestations are generally milder than that found in hemophilia. The patients demonstrate excessive bruising, epistaxis and hematomas, but hemarthroses are rare. Excessive bleeding may occur after circumcision or other surgical procedures or trauma. Congenital hypofibrinogenemia apparently also occurs.

Acquired fibrinogen deficencies have been reported in a variety of conditions such as liver disease, shock, burns, leukemia, cyanotic heart disease, transfusion reactions and in complications of pregnancy.

In the hypofibrinogenemias, clotting is delayed, and the clot eventually formed is extremely small. Treatment of hemorrhagic episodes is dependent upon administration of blood, plasma or purified fraction I. Since bleeding apparently occurs only with fibrinogen levels below 60 to 80 mg. per 100 ml. (normal 250 to 350 mg.), an attempt should be made to achieve this concentration. It has been shown that in afibrinogenemia 50 per cent of the administered fibrinogen disappears in 48 hours after transfusion; the remaining fibrinogen disappears more slowly, so that an additional loss of 50 per cent requires four to five days. A dose of 100 mg. of fibrinogen per kilogram of body weight will raise the fibrinogen concentration of an afibrinogenemic patient to approximately 200 mg. per 100 ml.

CIRCULATING ANTICOAGULANTS

In various conditions hemorrhagic disease may be produced by development of circulating anticoagulants which impair the normal coagulation cycle. These may develop spontaneously, as a complication of transfusion therapy or other hemorrhagic states, or in association with a variety of diseases such as lupus erythematosus, periarteritis nodosa, tuberculosis, chronic renal disease, and after treatment with nitrogen mustards and radiation. In many instances the anticoagulants have been found to have characteristics of antibodies. The whole blood clotting time is usually prolonged, and the patient's blood or plasma may be demonstrated to be capable of prolonging the clotting time of normal blood.

SUMMARY

The rapid increase in knowledge and understanding of the coagulation mechanism has led to the realization that hemorrhagic disorders

are far more common than previously supposed. It has become possible to identify and classify the hemorrhagic diseases fairly accurately with present methods, and the general designation of a patient as a "bleeder" should be abandoned. Because many of the hemorrhagic disorders are congenital in origin, the pediatrician is most likely to encounter the patients first. Recognition of these children is usually easy when severe disease exists, but may be difficult in the presence of mild deficiencies, particularly since many normal children demonstrate easy bruising and recurrent epistaxis. When one considers that the mild forms of hemorrhagic disease often erupt after such common pediatric procedures as tonsillectomy and dental extraction, it becomes apparent that much remains to be done. It has become obvious that the so-called preoperative screening procedures of bleeding time and clotting time are inadequate to detect any but the most severe disease states. This is particularly true when clotting times are done by the capillary method. On the other hand, it is unrealistic to expect that tests such as prothrombin consumption, thromboplastin generation, and others, be done on every child coming to routine tonsillectomy. Simple, but accurate and definitive, screening procedures are urgently needed. In the meantime a careful history referable to unusual bleeding in the child and in the family remains the best screening procedure available to the physician.

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PEDIATRIC TRANSFUSION THERAPY

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The dangers inherent in the therapeutic administration of human blood have become a considerable concern to the physician. He is confronted by estimates of mortality as high as one per 1000 transfusions and even higher. Serum hepatitis is expected to follow every 200 separate donor administrations, with a staggering incidence reported of one in five recipients affected after the use of pooled plasma. Sepsis, hemolysis, congestive failure threaten each therapeutic endeavor. Even the outcome of pregnancies still in the future is his obligation today.

Transposing such information in terms of the physician's own institution and his own transfusion policy is a matter of some difficulty. Nevertheless such reaction statistics reflect all too often the limitations in follow-up imposed on the investigator, and the errors are likely to be those of underestimated risk. The reports also usually represent superior transfusion services and are, one suspects, too conservative for application in general. However and wherever defined, the risk of transfusion is too great to be ignored.

The physician is therefore compelled to define his indications in terms of such risk. Of course when life is immediately in the balance, there will be no resort to statistical deliberation. The patient will get all the blood needed to maintain life with as much speed as is necessary. Hepatitis may become a concern after survival is assured. In less pressing situations, however, the risk of transfusion must be weighed carefully against the risk of treatment without transfusion. Transfusion is indicated only when the danger of not transfusing exceeds the risk of transfusion. If after such consideration blood is not indicated, it is contraindicated.

BLOOD BANKING PROCEDURES

Part of the evaluation of the hazard of transfusion is the consideration of the blood banking facilities to which patient and physician are

subject. From the selection of donors for typing and cross-matching to the supervision of the final administration there are factors to be weighed as to the risk of therapy. When one considers that the commonest cause of a fatal hemolytic reaction appears to be the transfusion of the wrong donor blood, the significance of even what appears to be too obvious to be of concern becomes frightening.

It is certainly impractical for any blood bank today to adopt all the procedures recommended in the interest of safety. Some techniques are still of unproved validity, and others have been supplanted by superior methods. Still others are useful so seldom that their incorporation into a blood bank regimen might threaten the performance of a more important operation. It would be presumptuous, however, in view of all the recent progress in banking, to assume that greater safety is generally to be found in fewer procedures. Blood is a "strange fluid." Its complexity, diversity and variability are unapproached by any other biological. So too are the problems of its administration. A blood bank today must be measured by the extent it is capable of adopting, and its reliability in performing, a multiplicity of precautionary practices. In this interest, the American Association of Blood Banks has published a manual summarizing present opinion as to techniques and safeguards of blood bank operation which deserves the greatest dissemination.[1] Some aspects of banking meriting the clinician's attention especially are the following:

Hepatitis

There is as yet no means of insuring the absence of live hepatitis virus from donor blood. Although eliminating donors with a history of jaundice or even recent transfusions may reduce the incidence of virus contamination, there is still the problem of the asymptomatic virus carriers. Liver "function" tests on donor blood such as thymol or zinc turbidities can similarly be expected to be of no more than a partially effective screen. Nevertheless this disease is so important a complication of blood transfusion as to warrant the welcome of any and all steps likely to limit its transmission.

The danger of acquiring hepatitis is in proportion to the number of donors used for transfusion or the number of individuals contributing to a pool, as in the case of plasma. A child receiving blood from two donors is exposed to twice the risk it would if both transfusions came from the same bottle. In this respect, pooled plasma represents the risk of a great many transfusions. There is yet no practical method of inactivating the virus (or viruses) in whole blood or fresh, frozen or lyophilized plasma. Although experimental observations had favored the use of ultraviolet irradiation for this purpose, clinical trials with

irradiated pooled plasma have been discouraging. Heat-treated albumin solutions, gamma globulin preparations and plasma aged for six months at room temperature appear at present to be largely free of these agents.

Blood Groups

Ideally, a patient should be given blood only of a corresponding blood type. The danger of hemolysis would then almost be eliminated and there would be no risk of isoimmunization except for unknown factors. Practically, this seems unattainable in view of the multiplicity of blood groupings, scarcity of many typing serums and obvious donor and storage limitations. Even the demands for comparable major blood types may often be exceeded, and in the urgency of the moment the following compromises made:

Group O donor blood given to group A or B or AB patients;
Group A donor blood given to group AB patients;
Group B donor blood given to group AB patients.

These compromises are predicated on the compatibility of the recipient's plasma for the donor's cells and the assumption that the donor's plasma will be so diluted on transfusion to the patient as to become innocuous. The latter is not always true. Some donors have potentially "dangerous" antibodies in sufficiently high titers as to be capable of destroying the recipient's red cells. Reactions following the administration of such blood may vary from mild and clinically insignificant hemolysis to a violent and possibly fatal transfusion reaction. Attempts to neutralize these antibodies by the addition of group A and group B specific soluble substances of animal origin offer incomplete protection at best. Not only do these group-specific polysaccharides react poorly with certain so-called immune antibodies of pronounced pathogenicity, but they also carry a risk of hyperimmunization. Whether such altered sensitization could be involved in the production of A-B-O hemolytic disease is a matter of conjecture.

The characterization of "low titer" blood as safe for such compromise transfusions also represents a practical delineation rather than an absolute one. Thus, although a saline agglutinin titer of the donor's antibodies for the patient's major blood group of not over 1:200 or 1:250 makes a transfusion catastrophe unlikely, it does not always protect against hemolysis of the patient's cells, especially on multiple administrations. Possibly, when there is more information on the value of hemolysin or incomplete antibody titers, this disadvantage will be minimized.

If blood of the same major group is not available and any of the

above compromises must necessarily be made, the following suggestions are made in order of preference:

1. Use packed or completely sedimented red cells whenever they would serve as well!

2. Use whole blood of low saline agglutinin titer against the patient's major blood group, i.e., less 1:200!

3. Use whole blood after the addition of group A and/or group B specific soluble substances!

Segregation of donor blood into Rh_o (D)-positive and the less common Rh_o (D)-negative types is now practically universal. Most banks are usually able to supply Rh_o-negative recipients with appropriate Rh_o-negative blood, although often after considerable difficulty. Exceptions are to be tolerated only in extreme emergency. To rest at this point, however, must be a matter of necessity and not intention. The incidence of isoimmunization to the subgroups of the Rh factor is not negligible. Both hemolytic disease of the newborn and transfusion reactions have resulted from such sensitization. Although the matching of these subgroups can hardly be accomplished more than occasionally, it must be recognized as an additional risk to subsequent transfusion or in a girl to childbearing. It is desirable that at least all the Rh_o (D)-negative blood be further categorized as to rh′ (C), rh″ (E) and Rh (Du) factors. The Rh (Du) factor can be especially troublesome because it behaves antigenically much as does the Rh_o (D) factor, even though it usually appears as Rh_o-negative in the ordinary typing procedures. It can readily be demonstrated by using the antihuman globulin technique (Coombs) after exposing the cells to potent anti-Rh_o serum.

Cross-Matching

The standard saline agglutination cross-match is little more than corroboration or denial of donor's and patient's main blood group compatibility. As such it has great merit. It will not demonstrate the incomplete antibody which characterizes almost all isoimmunization to Rh and Kell antigens. It should certainly not be considered "standard" if the patient was transfused a week or more before and has had time to develop antibodies in response to the former transfusion, nor should it suffice when transfusion is contemplated in the face of a previously uncharacterized antibody in erythroblastosis. In these circumstances assurance of compatibility in cross-matching comes only from the utilization of a method such as the "indirect" antihuman globulin test (Coombs), which can reveal the presence of incomplete antibodies. This test, when carefully performed, is highly sensitive and is applicable to almost all types of isoimmunization. Its adoption as a routine cross-match would make reliance on the history of previous transfusion

unnecessary. Unfortunately, it is time consuming and may have to be sacrificed to a quicker procedure when blood is needed urgently.

VOLUME OF TRANSFUSION

Commensurate with its importance, there is probably less investigative information available on the volumetric aspects of intravascular administration than on any other category of blood transfusion in pediatrics. This is notwithstanding the feelings of many that, in children, overloading the circulation in treatment of anemia and either overtransfusion or undertransfusion in surgery or hemorrhage account for a large portion of the deaths related to blood transfusion. Unfortunately these reactions are seldom recognized as such and less seldom recorded. The symptoms of cardiac decompensation or shock are usually assigned to the severity of the anemia or hemorrhage or the trauma of surgery and anesthesia rather than to an error in evaluating quantity and rate of administration. The clinician has been forced to rely on pediatric experience, with attending trepidations. The successful utilization of another physician's experience or advice implies the use of similar judgment. It is here that our communicative ability is frequently deficient. We establish guides to transfusion, but are forced to hedge as to limitations and the application to a specific circumstance. It is with full cognizance of this weakness that the following generalities are offered.

Maximal Transfusion

Although a standard pediatric transfusion can hardly be said to exist, the figure of "10 cc. per pound of body weight" is commonly associated with such a thought. Pediatric texts present such a value, and it is widely followed as a guide. Unfortunately, all too often the number is retained out of context. Qualifications such as "not more than approximately 10 cc." and "not more than 10 ml. per pound" are infrequently recalled, and little thought is given to its representation as a maximal pediatric transfusion.

It would probably be prudent to forget the 10 ml. as well as the limitation. Although many children withstand an increase in blood volume comparable to the transfusion of three pints to an adult, some do have tachycardia and others an increase in liver size and a gallop rhythm. Those requiring blood the most are often the least tolerant of such a quantity, as children with severe anemia or anemia associated with heart failure, high fever or serious infection. The manifestations of overloading are likely to be attributed to the complication and proper therapy not instituted. If transfusions are restricted to circum-

stances in which the risk is justified, few candidates are left to whom 10 ml. per pound can be administered safely.

The adoption of 5 ml. per pound as a maximal pediatric transfusion figure is strongly recommended. With the use of packed or sedimented red cells, hemoglobin increases are easily obtained comparable to those following the administration of 10 ml. per pound of whole blood. Excluding, of course, replacement in hemorrhage, it will rarely be desirable to exceed 5 ml. per pound in a single transfusion. It is usually given at a rate of 15 or 20 drops per minute. The figure can also be adapted to the administration of any intravascular colloid. If the patient's general condition or circulatory status is compromised, even as little as 3 ml. per pound should be administered, and this more slowly. It must be added here that the use of a "push" administration is often a matter of expediency for the physician and, with the questionable exception of the newborn, would seldom seem justified for the patient.

Chronic Anemia

The severity of anemia can serve as a guide to the volume of a transfusion. The basis is the observation that the more anemic the patient, the more likely that cardiac decompensation will be precipitated or aggravated by a further increase in blood volume. Less blood is therefore administered at one time to the severely anemic than to patients with moderate degrees of anemia despite the greater hemoglobin requirement of the former. The attempt to satisfy the more urgent requirement in one large single transfusion has resulted in fatality even though an occasional patient has had the reserve to withstand such an insult.

It has been the policy in the Children's Hospital of Michigan to consider the neighborhood of 5 gm. per 100 ml. hemoglobin concentration, more or less, as a demarcation between transfusion and more conservative therapy in uncomplicated chronic anemias. For example, an otherwise well infant with an iron deficiency anemia of 6 gm. per 100 ml. severity, who could have been followed reliably and closely, was treated with an oral iron preparation without transfusion. The advent of a significant infection may have altered the original decision in such a case, but, in general, a child in whom anemia has developed gradually was usually remarkably lacking in symptoms until its hemoglobin fell to somewhere about 5 gm. When some not-too-grave complication indicated that greater safety lay in transfusion, the child would tolerate administrations of 5 ml. per pound. On the other hand, infants or children with iron deficiency anemias of 3 gm. hemoglobin concentration or less were often either clinically decompensated or became so on slight provocation. The stress of a physical examination or the activity

involved in starting a transfusion has sometimes been sufficient to produce manifest heart failure. (Such severely anemic infants and apprehensive children are best sedated soundly and placed in oxygen with the head end of their beds elevated prior to the initiation of any extensive diagnostic or therapeutic effort.) Even the transfusion of 5 ml. per pound would occasionally result in decompensation. In these situations, restricting the transfusion volume to 3 ml. per pound, given at a rate of 10 drops per minute or less, was more uniformly satisfactory. Similar experience in the management of children with thalassemia,

TABLE 31. *Hemoglobin Values in Chronic Anemia as a Guide to Volume of Transfusion*

PATIENT'S HEMOGLOBIN	VOLUME OF TRANSFUSION
3 gm. % or less	3 ml. per pound
4 gm. %	4 ml. per pound
5 gm. % or more	5 ml. per pound

The milliliters per pound refers to a generally safe increase in blood volume and applies to whole blood, packed or sedimented red blood cells, plasma or albumin solutions.

TABLE 32. *Approximate Hemoglobin Increases after Transfusion by Weight*

VOLUME OF TRANSFUSION	APPROXIMATE HEMOGLOBIN INCREASE AFTER	
	Whole Blood	Sedimented Cells
3 ml./lb.	1 gm. %	2–2.5 gm. %
4 ml./lb.	↕	↕
5 ml./lb.	1.5 gm. %	3–4 gm. %

These values are grossly approximate 24 to 48 hours after the transfusion. Variations are considerable because of differences in hemoglobin concentration of donor blood and rates of plasma volume adjustment in the recipient.

sickle cell disease and other chronic anemias has seemed to justify a transfusion guide between the limits of 3 and 5 ml. per pound.

Table 31 illustrates this guide: A patient who has a hemoglobin concentration of 3 gm. per 100 ml. or less is given a transfusion of 3 ml. per pound of body weight. Anemia of 4 gm. is countered with the administration of 4 ml. per pound, and so too for 5 gm. or higher with 5 ml. per pound of body weight. It has been referred to as the 3-4-5 rule. Three milliliters per pound is considered a minimum transfusion, and 5 ml. per pound the maximum. Rates of administration vary between 20 drops per minute as an arbitrary standard to as little as 10 drops per minute in more precarious situations. Any complication compromising the circulatory status results either in transfusion of less blood than the guide indicates or administration at a slower rate, or both. Hemo-

globin increments expected after the transfusion of these quantities are presented in Table 32.

The formula is applied equally well to the transfusion of packed or sedimented cells or whole blood. Sedimented red blood cells are the treatment of choice, however, for the correction of anemia. Transfusion may be repeated in 24 hours and occasionally as soon as 12 hours after the initial procedure.

If an anemic patient is in severe decompensation at the time of presentation to the physician, any increase in blood volume may prove disastrous. Therapy in such situations should consist in raising the patient's hemoglobin and decreasing the blood volume simultaneously. This can be accomplished by a brief exsanguination transfusion. Even offering as little as 3 to 5 ml. per pound packed or sedimented red cells while removing a somewhat larger amount of blood at the same time can be lifesaving. Although the procedure is easily performed in the newborn period when the umbilical vein is still patent, it becomes a formidable endeavor afterward. Adequate sedation is paramount in an older infant or child lest the exertion and trauma of the operation offset the benefits. Fortunately the circumstances requiring such effort are uncommon.

Acute Anemia

In hemolytic anemias of rapid progression the guide is not so reliable. Although the circulatory principles still obtain, the patient must be followed closely, and liberties with numbers, rates of administration and frequency are often taken. Hemoglobin levels as low as those tolerated in the chronic anemias are dangerous, and the physician should set his sights a good deal higher.

Acute Hemorrhage

For transfusion purposes, acute hemorrhage may be defined as bleeding that occurs too rapidly for the body to maintain its blood volume. The net result is a decrease in the vascular compartment with symptoms according to severity. Treatment is based on (*a*) control of hemorrhage, (*b*) signs of shock, (*c*) degree of anemia.

If the bleeding is controlled or readily controllable, if the child's general status is good and there are no signs of shock, and if hemorrhage is not likely to recur, transfusion is not indicated. Anemia is usually not important under such circumstances (in acute hemorrhage), and the patient may be encouraged to regenerate his own blood. When iron therapy will do, it is safer than transfusion.

If, on the other hand, hemorrhage is not immediately controllable

and is severe, volume for volume replacement by whole blood is required. The physician is guided not only by his estimates of blood lost, but also by repeated appraisal of the patient's pulse rate and blood pressure. It is important to watch for an increase in pulse rate as an early sign of decreased blood volume. By the time hypotension occurs, the patient may be severely handicapped. In a situation of more or less continuous blood loss and replacement, tachycardia may also be an indication of cardiac decompensation due to overloading. Skin color, pulse quality, blood pressure and auscultation of the heart will usually encompass a differentiation. When massive transfusion or artificial circulation is anticipated, as in vascular or cardiac surgery, heparinized blood or blood collected through an ion exchange column is recommended to avoid citrate toxicity. Blood so collected must be used promptly, since storage impairs it. Thrombocytopenia should also be anticipated when a great many transfusions are given in a short time.

Protracted Hemorrhage

If protracted hemorrhage is considered to be blood loss at a rate slow enough to permit the body's maintenance of its own blood volume, there will be no signs of shock, and symptoms will depend on the severity of the anemia. Repeated or continuous loss of small quantities of blood will eventually result in a clinical and hematological picture of iron deficiency. When this occurs, it is usually referred to as chronic blood loss. Despite the etiology of their anemia these children may tolerate increases in blood volume poorly. The treatment is essentially that of anemia. The greater the chronicity of the hemorrhage and the more pronounced the anemia, the less transfusion volume should be offered.

Combinations of acute and protracted hemorrhage occur, and the physician must evaluate the requirements of anemia and decreased blood volume separately. Whole blood or packed red cells are then used accordingly.

SELECTIVE TRANSFUSION

The transfusion of whole blood is giving ground to the administration of separate portions for specific purposes. Packed or sedimented red cells are being used preferentially in the treatment of anemia. Washed red cells can be easily prepared if a plasma reaction is feared. Fresh, frozen, type-specific or pooled plasma is available. Lyophilized pooled plasma products are offered commercially with or without expressed antihemophilic potency. Protein fractions such as fibrinogen,

albumin and gamma globulin are meeting wide acceptance. Even platelet preparations are now within the scope of most blood banks.

It is a good trend. Admitting the irreplaceable value of whole blood in the treatment of hemorrhage, we must also concede its disadvantages in the therapy of other conditions. Hemoglobin values can be raised more efficiently if the plasma is removed. More plasma can be given with less danger of hemolysis if red cells are excluded. Heat-treated albumin and gamma globulin preparations, when called for, offer less risk of hepatitis, and so on. These derivatives enable the physician to treat not only more effectively, but more safely as well. There is an additional advantage. The multiplicity of blood bank products encourages thinking in terms of the patient's specific requirements and bids fair to remove blood from the category of "shotgun therapy."

Some commonly used blood products are as follows:

Packed or Sedimented Red Blood Cells

Packed or sedimented cells are easily prepared in almost any banking facility. A donor bottle is chosen in which the red blood cells have settled by gravity and are delineated from the supernatant plasma. Centrifugation may be utilized to increase the hemoglobin concentration of the preparation if appropriate apparatus is available. The plasma is removed only immediately prior to administration, great care being taken to preserve asepsis. First the vacuum is released by means of a needle with an air filter. The hole to the glass air vent tube must not be punctured, or the cells will be resuspended. The plasma is then siphoned into a sterile vacuum bottle by means of a long metal tube sold especially for this purpose. After suctioning, both needle and tube are withdrawn and the cells administered through a regular blood filter. In emergency the red cells can also be removed by an ordinary needle through the glass air vent tube present in the bottle. The former method is preferred, since it avoids additional mechanical injury to the red cells. Once the donor container has been re-entered, it is inadvisable to store the cells for any length of time because of the added opportunity for bacterial contamination.

Plastic transfusion equipment is also easily adapted for red cell preparations. This can be accomplished without resorting to long metal tubes or vacuum bottles.

Platelet Preparations

Platelets obtained by ordinary methods of blood collection are largely incapable of surviving in the blood of a recipient. If precautions are

taken to collect blood by gravity rather than by vacuum, if the blood is exposed to nonwettable surfaces such as silicone or plastic only, and if it is administered promptly after collection, transfusion of viable platelets is practical. The vehicle may be the whole blood in which the platelets are collected or, if a greater concentration is desired, it can be obtained by differential centrifugation. The greater the degree of manipulation, however, the more likely that platelet damage will offset the benefits of concentration. Both platelet storage and effective platelet derivatives have been achieved experimentally, suggesting a broadened field of therapeutic application in the future.

At present the indications for platelet administration in pediatrics are those of hemorrhage or necessary surgery in the face of severe thrombocytopenia. The transfusion of children suffering from uncomplicated thrombocytopenia with either blood or a platelet preparation may result in the formation of antiplatelet antibodies capable of adversely affecting treatment at a more critical time. The detection of such antibodies is probably too difficult a procedure for most blood banks.

Plasma

Plasma is still the mainstay of therapy for the coagulation disorders. Fresh, frozen, lyophilized or ordinary blood bank plasma can be used according to the nature of the deficiency and the availability of the product. Lyophilized pooled plasma can be obtained commercially and has excellent storage qualities. Unhappily, it carries a greatly increased risk of hepatitis. Repeated and massive administration of untitered lots of pooled plasma is also potentially dangerous with respect to hemolysis. After such use, recipients of blood groups A_1 or B or AB may have a severe hemolytic anemia due to the anti-A or anti-B antibodies in the pool. It is hoped that lyophilized plasma will soon be produced in individual type-specific units.

The use of plasma as an emergency blood volume expander or for maintenance of nitrogen balance will probably diminish as facilities for manufacture of albumin solutions expand. Heat-processed albumin carries neither the risk of hemolysis nor the same danger of hepatitis as does plasma.

TRANSFUSION REACTION

The recognition and management of transfusion reactions should follow any discussion of transfusion therapy. Although space limitations preclude inclusion here, the subject in general has been excellently summarized in recent publications.[2, 3]

CONCLUSION

Blood transfusion is indicated only if the risk of withholding blood is greater than the danger of transfusion itself. If transfusion is not indicated, it is contraindicated.

A maximum pediatric transfusion of 5 ml. per pound of body weight is suggested, and a guide presented relating volume of acceptable transfusion to hemoglobin values in chronic anemia.

The administration of portions or fractions of whole blood is encouraged as a means of more effective treatment as well as decreased risk.

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POLIOMYELITIS, 1957

The Pediatrician's Role in Poliomyelitis Prevention

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The role of the pediatrician in the prevention of paralytic poliomyelitis is an important and continuing one. Though all physicians in the United States may be properly concerned with the present nationwide campaign to vaccinate everyone up to age 40 against paralytic poliomyelitis, the protection of the oncoming generations for years ahead falls particularly in the province of the pediatrician. It is a responsibility which he shares most immediately with the general practitioner and the obstetrician.

The challenge that faces the pediatrician in the prevention of paralytic poliomyelitis is nothing new to him. Pediatricians have been responsible for the success of immunization programs generally. They have taken leadership not only in performing immunizations, but also in impressing their medical colleagues with the value of such programs and in encouraging families to seek immunization protection. The low and declining level of disease against which immunization affords protection, notably diphtheria, whooping cough, tetanus and smallpox, attests the success of the pediatrician's leadership in this important phase of preventive medicine.

At present, and until the huge backlog of unprotected people has been vaccinated, the role of the pediatrician in the control and eventual elimination of paralytic poliomyelitis is threefold. He is first of all a clinician, concerned with vaccinating his own patients and their families against the now needless threat of this disease. His second role is that of an advocate within the councils of organized medicine. Be-

From the National Foundation for Infantile Paralysis, New York City.

cause of his particular concern for the practice of preventive medicine, the pediatrician is in a favorable position to urge the participation of his medical colleagues in community programs established to eliminate the risk of paralytic poliomyelitis. Thirdly, the pediatrician must stand out as a responsible citizen of the community. Not only his own immediate patients, but also other members of the community where he practices, look to him for leadership in establishing, supporting and operating community programs for the early and continuing control of paralytic poliomyelitis. Clinician, advocate and citizen, the pediatrician is all three. The information presented in this article has been selected in the hope that it will help the pediatrician play his threefold role.

We shall make no attempt to instruct the pediatrician in his role as citizen. It is also recognized that every man must decide for himself whether he will be the advocate of any cause, however worthy. In the matter of prompt and early vaccination against paralytic poliomyelitis, the pediatrician advocates it by practicing it. How much further he wishes to go—in verbal and organizational advocacy—must remain a matter of individual interest and conscience.

AN EDUCATIONAL PROBLEM

The problem of poliomyelitis prevention has become perhaps more an education problem than a medical problem. Organized medicine has recognized its responsibility for helping to solve this problem.[10] On January 26, 1957, the American Medical Association convened a special Conference on Poliomyelitis Vaccination in Chicago. To delegates assembled from every state Dwight H. Murray, M.D., president of the American Medical Association, said, "I implore and urge you to do everything possible to get your state and county medical societies behind the A. M. A. polio campaign to encourage everyone to be vaccinated up to age 40."

The anticipated role of the pediatrician in organized medicine's campaign to eliminate paralytic poliomyelitis was summed up by Edgar E. Martmer, M.D.,[9] president of the American Academy of Pediatrics, who said, in part, at the January 26 meeting:

> On behalf of the American Academy of Pediatrics, I am happy and proud today to pledge our complete and enthusiastic support in this total war against paralytic polio. . . . Poliomyelitis will cease to be an important health hazard only when all the population under 40 years of age is protected. . . . The fact remains that less than one-third of the injections necessary to eliminate paralytic polio have been given. We are still 200 million shots short of winning the war against this disease.
>
> If we are to conquer paralytic polio, . . . then each of us . . . must encourage our patients to be immunized themselves and to have their entire families immunized . . . Paralytic polio can be licked. We can and must do it.
>
> The 5,000 members of the American Academy of Pediatrics recognize their immediate responsibilities and will do their utmost to assist in any and every way possible.

PARENTS NEED PROTECTION TOO

The importance of the pediatrician in the nation's poliomyelitis vaccination program during the present emergency campaign and in the long future is well recognized outside the specialty. When Jack C. Haldeman, M.D.,[4] chief of the Division of General Health Services of the U.S. Public Health Service's Bureau of State Services, addressed the American Academy of Pediatrics in New York in October, 1956, he said:

> No group has a more important role in achieving full protection against poliomyelitis than the pediatricians of this country. More mothers are taking their children to pediatricians than ever before. Taking full advantage of this opportunity is a real challenge.
>
> Pediatricians have an excellent opportunity to urge young parents to seek immunization for themselves as well as for their children. The incidence of poliomyelitis among young parents is also high, and the degree of paralysis severe, often with tragic socio-economic consequences.

Doctor Haldeman also suggested that pediatricians may have a role in vaccinating teen-agers, who are slightly over the age of patients they usually see. "Perhaps the older siblings of the pediatrician's patients could be offered immunization if they are not being reached by other arrangements," Dr. Haldeman suggested.

THE NATIONAL PICTURE OF POLIOMYELITIS VACCINATION

The physician who advocates an "all-out" vaccination campaign in his own community must be fortified with local facts. A national campaign is nothing more than the sum of local efforts. Yet in assessing local efforts it may be useful to compare them with over-all information, compiled on a national basis. The urgency of the 1957 poliomyelitis vaccine drive is clearly exhibited in nationwide figures. These are summed up in Table 1.

The heart of the problem is that, as of March 1, 1957, there were 58,000,000 people under 40 in the United States who had received no vaccine at all. Only 10 per cent of the group under 40 had received a full immunization schedule of three doses of the vaccine. Most of the vaccinated in this group are under 10 years of age or recent mothers.

Not enough headway has been made in vaccinating young adults. Vaccinations among preschool children have also lagged. In fact the U.S. Public Health Service estimated that in 1956 about 39 per cent of all cases of paralytic poliomyelitis occurred in children under five years of age and 34 per cent among patients 15 years and older. A greater severity of paralysis with tragic personal consequences is usually found in the older patient. Only about 2 per cent of the cases of paralytic poliomyelitis occur after age 40.

It is another matter of interest that most of those who have so far received vaccine were vaccinated in clinics established for this purpose.

TABLE 1. *Estimated Number of Persons and Percentage of U. S. Population with 1, 2 and 3 Doses of Salk Vaccine, by Age Group, March 1, 1957*

VACCINATION STATUS OF U.S. POPULATION	TOTAL ALL AGES	UNDER 40	UNDER 20 AND PREGNANT WOMEN	20–39, EXCLUDING PREGNANT WOMEN
Population	170,000,000	109,000,000	67,000,000	42,000,000
Number with:				
One dose	13,000,000	13,000,000	9,000,000	4,000,000
Two doses	27,000,000	27,000,000	25,000,000	2,000,000
Three doses	11,000,000	11,000,000	10,000,000	1,000,000
One or more doses	51,000,000	51,000,000	44,000,000	7,000,000
No vaccine	119,000,000	58,000,000	23,000,000	35,000,000
Percentage with:				
One dose	8	12	13	10
Two doses	16	25	38	5
Three doses	6	10	15	2
No vaccine	70	53	34	83

Based on data compiled by National Foundation for Infantile Paralysis and U.S. Public Health Service.

VACCINE PROTECTS ONLY THE VACCINATED

The drive toward universal vaccination with poliomyelitis vaccine is based upon epidemiological findings. Though the Salk vaccine continues to show a high degree of effectiveness in the prevention of *paralytic* poliomyelitis, considerable evidence has accumulated to show that it is of little, if any, value in preventing nonparalytic cases and in controlling the spread of poliomyelitis virus. It must be pointed out, however, that there has been as yet but limited time to evaluate the effectiveness of a full three-dose schedule of vaccine.

Apparently, however, poliomyelitis vaccine differs from smallpox vaccination and diphtheria immunization in controlling the spread of disease. If a critical percentage of a population is immunized against smallpox or diphtheria, the epidemic disease can be reduced to a vanishing point. But this does not appear to be so with poliovirus infection. We cannot expect that even universal vaccination against poliomyelitis—with any type of vaccine—will eliminate the poliovirus from the population. The example of Egypt can be cited. In that country nearly everyone over two years of age has a natural immunity to poliomyelitis through actual exposure to the disease. Yet the poliovirus itself remains widely spread through the country. It is illogical to suppose that if natural immunity cannot expel the poliovirus from a population, vaccination can achieve this goal.

The conclusion to be drawn from the epidemiological facts is simply this: No one can be protected against paralytic poliomyelitis by somebody else's vaccination. Those who go, or are left, unvaccinated probably continue with just as great a risk of contracting paralytic poliomyelitis as if no vaccine existed.

SUMMARY OF 1956 EXPERIENCE

The hope of controlling paralytic poliomyelitis now depends upon the widespread use of the Salk inactivated virus vaccine. The speed with which this task is performed will determine the date when paralytic poliomyelitis will be virtually eradicated. In the United States the target date has been set for the summer of 1957. The cooperation of pediatricians is essential to hitting this target. Pediatricians are taking the lead in this as in other campaigns of immunization.

The incidence of poliomyelitis has been declining in recent years, after hitting a peak in 1952. In 1956 a total of 15,400 cases was reported. This was a decline of 47 per cent from the previous year and of 59 per cent from the five-year average number of cases for the years 1951–5. The sharpest drop in paralytic cases occurred in the age group five to nine, which has been most extensively vaccinated.

All reported experience with the Salk vaccine in 1956 confirms and extends previous reports of its safety and efficacy. Collecting and disseminating information on exactly these points is the function of the National Poliomyelitis Surveillance Program, established in April, 1955, at the Communicable Disease Center of the U.S. Public Health Service. A preliminary report on the surveillance of poliomyelitis in the United States in 1956 has been published.[8] A few of the highlights of this report may be summarized as follows:

Over 70 million inoculations of vaccine were given during 1956. Several hundred cases of poliomyelitis occurred shortly after inoculation, but this many vaccine-associated cases would be anticipated as purely coincidental. There was slight, if any, evidence of untoward reactions from the vaccine. If there were any hypothetically unfavorable effects of the vaccine in 1956, they occurred at a frequency of less than one per million inoculations.

The effectiveness of the vaccine in preventing paralytic poliomyelitis was calculated from a number of independent studies to stand at a level of about 75 per cent, even though a large proportion of the vaccinated population had received less than the recommended schedule of three doses.

The Salk vaccine is not 100 per cent effective. But studies to date suggest that the full course of three properly spaced doses may be better than 90 per cent effective against paralytic poliomyelitis. There

are a number of indications that vaccination tends to modify the severity of paralytic involvement should it occur.

DOSAGE SCHEDULES

The practical dosage schedule for the Salk vaccine is now well standardized, as follows: two 1-cc. intramuscular injections, spaced two to six weeks apart, with a third (booster) injection, also 1 cc., given at least seven months after the second injection.

Dosage schedules and antibody responses have been the subject of continuing research on the part of Dr. Jonas E. Salk[11, 12, 13] at the University of Pittsburgh. Recommendations for the use of the vaccine are based largely on his studies.

Although it is desirable to adhere to the dosage schedule cited above, it should not be regarded as sacrosanct. Though longer intervals between injections may produce even higher antibody levels, a reasonable booster effect occurs as early as three months after the second dose of vaccine. Certainly in the face of a sharply rising incidence of paralytic poliomyelitis in a community—an epidemic threat—the third, or booster, shots should be given earlier than the usual seven months' interval.

The greater risk of paralytic poliomyelitis in the pregnant woman is well known, and pregnancy is an indication for vaccination. The further advanced the pregnancy, the greater the chance and perhaps the severity of paralytic involvement. Maternal vaccination has the additional desirable effect of conferring a temporary passive immunity on the infant. A booster dose of vaccine early in pregnancy would seem well warranted, for the benefit of the previously vaccinated woman as well as for the additional protection to the infant.

At present the first injection of poliomyelitis vaccine is usually given at about six months of age. A few studies of immunization at earlier ages have been made, and others are in progress. Brown[1] and Brown and Smith[1, 2] reported inconclusive results in a group of vaccinated infants between two and 12 months of age; the vaccine used was later reported to be of low potency. Batson and Christie,* however, have obtained most encouraging results in their studies of responses of infants from birth to six weeks of age to poliomyelitis vaccine alone, to poliomyelitis vaccine combined with DPT (diphtheria, pertussis, tetanus) antigens, and to poliomyelitis vaccine and DPT antigens given simultaneously, but at a different site. Their work will soon be reported.

COMBINED ANTIGENS

The highly practical question of combining poliomyelitis vaccine with diphtheria, pertussis and tetanus inoculations has, of course, arisen.

* R. Batson and A. Christie: Personal communication.

A number of studies on the use of combined antigens are now in progress. Theoretically, there seems to be no reason why the combination of antigens should adversely affect the response to any of them. However, the practice of administering diphtheria, pertussis and tetanus antigens *in the same syringe* with poliomyelitis vaccine cannot yet be recommended. It has not yet been determined that the combination of the four antigens will give as good antibody response as the administration of the DPT immunization and the poliomyelitis vaccine separately. At present, if given at the same time as DPT immunization, poliomyelitis vaccine should be injected at a different site, Salk states.

It may be predicted that combined antigens, including poliomyelitis vaccine, will be used. Every pediatrician, parent and child will appreciate the advantage of such a product when it becomes available. It must be pointed out, however, that the product is not now on the market, and there is no justification for postponing or delaying immunizations with the separate poliomyelitis vaccine and other immunizing agents presently on hand.

When combined poliomyelitis vaccine and DPT antigens become available, this should have the favorable effect of encouraging more widespread use of immunization procedures generally. Health authorities concerned about lagging immunization rates, as evidenced by recent diphtheria outbreaks, will welcome a product that combines poliomyelitis vaccine with DPT antigens.

No one need go without vaccination for fear of penicillin reaction. This risk in general has proved to be minimal. One manufacturer, however, now produces a vaccine with only a minute trace of penicillin, and this product can be used in patients known to be exquisitely sensitive to penicillin.

DURATION OF IMMUNITY WITH SALK VACCINE

Another important clinical question is the duration of immunity following a full, properly spaced series of three injections of Salk vaccine. In other words, will additional booster shots be necessary and, if so, when should they be given? This question cannot be finally answered until sufficient time has elapsed to put it to positive clinical test. The present indication is that the immunity, as judged by hyperreactivity to subsequent antigenic stimulation, tends to persist. So far the persistence of hyperreactivity has been observed to endure for a little over three years. It may be anticipated that individual differences in immune responses, and their duration, will be encountered.

Measurable levels of antibody in the circulating blood have commonly been taken as the criteria for determining effective response to poliomyelitis vaccination. However, as Salk has pointed out, an immunologic hyperreactivity continues even when antibodies have de-

clined to nonmeasurable levels. This established hyperreactivity is apparently capable of invoking a rapid antibody response when needed. When challenged by intestinal or pharyngeal invasion of poliomyelitis virus, the established immunologic hyperreactivity apparently acts to induce the formation of antibodies with sufficient speed and in large enough quantity to intercept virus invasion of the central nervous system.

"POLIOMYELITIS-LIKE DISEASES"

There are an increasing number of reports of poliomyelitis-like diseases that would have been called poliomyelitis a few years ago. These diseases have been pinned down through tissue culture studies in the laboratory. Among the etiologic agents identified have been Coxsackie and ECHO viruses. ECHO is the abbreviation for enteric, cytopathogenic, human orphan virus. A number of strains of ECHO viruses have been discovered. The National Foundation for Infantile Paralysis is now supporting research to determine which of the ECHO viruses cause disease, particularly illness confused with poliomyelitis. The National Foundation has arranged for the preparation of type-specific antiserums against all the known types of ECHO viruses, so that they may be identified when encountered.

The poliomyelitis-like diseases are usually diagnosed as aseptic meningitis. This diagnosis was originally made in cases that exhibited the symptoms of meningitis, but in which no bacteria could be isolated.

Clinically, there is no difference between aseptic meningitis and *nonparalytic* poliomyelitis. The diagnosis is usually made on epidemiological grounds. During the poliomyelitis season, and with known cases of poliomyelitis in the community, the diagnosis of poliomyelitis is usually made. At other times of the year the same syndrome might be designated as aseptic meningitis. The accurate diagnosis requires laboratory evidence. A stool sample run through a series of diagnostic tissue cultures will give evidence as to whether any virus recovered is a poliomyelitis virus or something else. Virus laboratories have now been established in a great many state health departments, or are available through health departments to examine stool specimens submitted by physicians.

The accumulation of a considerable number of virus laboratory reports on poliomyelitis-like diseases, simulating the early symptomatology of poliomyelitis, leads to the impression that a high percentage of the cases diagnosed as nonparalytic or abortive poliomyelitis are in fact not caused by the poliomyelitis virus.

The epidemiology of Coxsackie and ECHO virus infections follows closely that of poliomyelitis itself—a high concentration of cases in the

summer months, predominantly among younger age groups. Any of these virus infections may produce the aseptic meningitis syndrome.

ECHO Virus Infection

Karzon, Barron, Winkelstein and Cohen[5] reported the isolation of ECHO virus type 6 during a seasonal outbreak of aseptic meningitis in the village of Holland, New York. Seven patients from this village were admitted to the Buffalo Children's Hospital in July, 1955, with a diagnosis of nonparalytic poliomyelitis. Laboratory observations included detailed virus studies of cerebrospinal fluid, blood, throat swabs and feces. In none of these patients was a rise in poliovirus neutralizing antibody demonstrable. In all cases there were rises in circulating antibodies to ECHO type 6 virus. At the time the patients were admitted to the hospital the disease present could not be distinguished clinically from preparalytic poliomyelitis. However, the patients improved rapidly and were all discharged within three to 10 days with no residual damage except occasional, slight muscular weakness and hamstring spasms. A total of 24 cases of aseptic meningitis were noted in Holland in July, 1955.

The association of ECHO virus type 6 with outbreaks of aseptic meningitis, as reported by Karzon and associates, does not seem to be an isolated phenomenon. Kibrick, Melendes and Enders[6] reported a high recovery rate of orphan viruses, largely ECHO virus type 6, during an outbreak of cases diagnosed as nonparalytic poliomyelitis in Massachusetts in the summer of 1954. Dalldorf noted the same thing in an epidemic of aseptic meningitis in Troy, New York, in the summer of 1955. Davis and Melnick[3] associated the recovery of ECHO virus type 6 with 24 cases of aseptic meningitis.

Coxsackie Virus Infection

Evidence that Coxsackie viruses, widespread in nature, may play an etiological role in aseptic meningitis has also accumulated. Three groups of investigators have isolated Coxsackie viruses from the cerebrospinal fluid. Kirby and Evans[7] described a group of patients, with a clinical diagnosis of poliomyelitis, in whom a relatively large percentage of the infections were apparently caused by Coxsackie group B viruses. Tissue culture methods were used for primary isolation and serologic identification of the virus.

Kirby and Evans studied 66 patients admitted to the King County Hospital, Seattle, Washington, from July through December, 1954, with a diagnosis of paralytic or nonparalytic poliomyelitis. Virus was obtained from stools of 51 patients. In 37 patients the virus strain

isolated was a poliovirus. In 14 patients it proved to be a Coxsackie B strain. The stools of one patient first yielded Coxsackie virus and later poliovirus. The clinical course of the patients from whom the Coxsackie virus was isolated was similar to that observed in nonparalytic patients from whom poliovirus was recovered. These patients became asymptomatic in a week to 10 days and had no residual paralysis.

Additional evidence will doubtless be presented associating Coxsackie and ECHO viruses with aseptic meningitis, nonparalytic poliomyelitis and poliomyelitis-like diseases.

AN IMPORTANT RESEARCH PROBLEM

One of the most important directions of research in relation to poliomyelitis is the search for stable strains of normal tissue that can be propagated indefinitely in tissue culture. The reason for this earnest search is that such tissue may some day be necessary to assure an adequate supply of poliomyelitis vaccine. The manufacture of the Salk killed-virus vaccine requires monkey kidney tissue as the medium upon which poliomyelitis virus is cultured and grown. A considerable supply of monkey kidneys is needed to assure large quantities of vaccine for mass immunization programs. The monkeys are imported from India. There is a risk that the supply of these monkeys will gradually but naturally diminish, and a further risk that their importation may be cut off by political fiat. There is also a question as to the availability of monkeys other than those imported from India to supply the large amount of kidney tissue necessary for vaccine manufacture.

Numerous strains of human cancer cells, like the HeLa cell, can be propagated indefinitely in tissue culture. No one knows what the hazards of using such cells for vaccine production might entail, and their use for this purpose is not proposed. However, HeLa cells have been of great usefulness for diagnostic tissue culture purposes.

Cell lines originating from normal tissue have also been carried through many generations of tissue culture. However, at the point at which they show the possibility of being propagated indefinitely, the question arises as to whether they have then become malignant. It may be that the ability to propagate indefinitely is itself a characteristic of malignancy in the cells so far under experimental investigation. This interesting question was discussed at a conference on cellular biology, nucleic acids and viruses, held in New York in January, 1957.

There are two fundamental possibilities for escaping a shortage of monkey kidney tissue that might prevent the manufacture of adequate quantities of the Salk killed-virus vaccine. One is the discovery and development of stable strains of normal human tissue in which the poliovirus can be grown and which will propagate indefinitely. The

other is the development of a clinically safe, live-virus vaccine. The quantity of virus necessary to make a live-virus vaccine is considerably smaller than the amount necessary for a killed-virus vaccine. The ratio may be as high as 1 to 1000. The live-virus vaccine would therefore offer tremendous advantage in that it would require only 1/1000 as many monkey kidneys for manufacture as Salk vaccine.

However, *all live-virus vaccine research is still in the experimental stage*. Albert Sabin, M.D., one of the principal investigators in this field, has expressly pointed out that the Salk killed-virus vaccine is the only vaccine now available for clinical use. The mass immunization programs now being fostered all contemplate the use of Salk vaccine.

SERVICES FOR POLIOMYELITIS PATIENTS

The development of the Salk vaccine promises future protection against paralytic poliomyelitis to those who avail themselves of it. The value and need of wide-scale vaccination programs may be emphasized by brief reference to the patients for whom a vaccine comes too late. The National Foundation for Infantile Paralysis is using and will continue to use its best efforts to encourage the widest possible vaccination against paralytic poliomyelitis. But the National Foundation will not abate its concern for those who have been stricken by the disease. In less than two decades the local chapters of the National Foundation have expended more than a quarter of a billion dollars for care of poliomyelitis patients.

It has become increasingly evident that there are possibilities for the fuller rehabilitation of patients stricken with paralytic poliomyelitis. In other words, we now know that more can be done for more patients than was previously thought possible. It seems worth while, therefore, to call attention to several programs supported by the National Foundation which are especially concerned with the rehabilitation problem. For some years the Foundation has been making grants to medical schools to teach the concepts and techniques of rehabilitation. Since 1950 it has supported the establishment and operation of respiratory and rehabilitation centers devoted to the most severely damaged of poliomyelitis victims. This year it is encouraging local community surveys of postpoliomyelitis patients to assist them more fully toward normal living.

Respiratory Centers

A full discussion of the respiratory and rehabilitation center program of the National Foundation would be a volume in itself. The following few facts must suffice. There are 15 such centers now operated in the

United States, all connected with medical schools. Admission of patients is at the discretion of each center. The fundamental goal of the centers is to demonstrate how much can be done to rehabilitate the most severely damaged of all patients with chronic illness—that is, the respiratory poliomyelitis case. To this end every center engages in research and teaching as well as patient care. Over 4000 patients have been admitted to the centers since their inception. Of those admitted in respirators, about half (48 per cent) have been discharged completely weaned from need for mechanical aids to breathing. The average stay in the centers has been about seven months. The research outcome of the center program has made great impact on the care of both acute and long-term poliomyelitis patients. The program as a whole has emphatically proved both the great humanitarian and economic advantages of providing "hopeless" patients with the most comprehensive patient care program that can be developed rather than consigning them to mere custodial care. It has been proved possible to discharge almost all such patients to successful home care programs, where the patients enjoy a happier life with their families and are often able to return to productive enterprise.

Community Surveys of Postpoliomyelitis Patients

The continuing home management of long-term poliomyelitis patients with residual crippling poses many problems to the physician in charge of the case. Often these problems are social rather than medical. Ideally, these problems should be handled by a well trained medical social worker. Unfortunately, such workers are in short supply. Many physicians may not be aware of the help available to them in dealing with these social problems from *volunteer* workers, properly selected for and oriented to this task.

The volunteer does not take the place of the professional worker. Nor is he or she involved in the medical aspects of the patient's condition.

Members of local chapters of the National Foundation for Infantile Paralysis have for many years offered a variety of personal services, on a volunteer basis, to patients stricken with paralytic poliomyelitis. The objective of these services has been to assist the crippled patient in attaining the fullest possible rehabilitation. The results of this volunteer service, as attested by many case histories, have been excellent. It has now been recommended to local chapters of the National Foundation that they survey and review with physicians the postpoliomyelitis cases in their own communities to find out their current medical and extramedical needs and to take steps toward meeting them. The basic pro-

cedure in the study is personal visitation, by appointment, to the homes of known patients. A certain number of these patients, experience shows, have lost contact with a physician, and others are not aware of the help available to them from community resources.

SUMMARY

The role of the pediatrician in the prevention of paralytic poliomyelitis is threefold—as clinician, advocate and citizen. If everyone in the United States up to age 40 is to be vaccinated against paralytic poliomyelitis at an early date, pediatricians must continue to take leadership in this program. The problem is as much an educational challenge as a medical opportunity. Parents need protection as well as children, and the pediatrician is in a unique position to reach the families of his patients. The epidemiological facts about poliomyelitis indicate that only those who themselves have been vaccinated are protected against the risk of paralytic involvement.

As of March 1, 1957, there were 58,000,000 people in the United States under age 40 who had received no poliomyelitis vaccine at all. Only 10 per cent of the under-40 age group had received a full schedule of three injections. The full course of injections may be better than 90 per cent effective in preventing paralytic poliomyelitis.

Experience with the Salk vaccine in 1956, when some 70 million injections were given, confirms and extends earlier reports of its safety and effectiveness.

The combination of poliomyelitis vaccine with diphtheria, pertussis and tetanus antigens is still in the experimental stage; but it may be predicted that combined antigens, including poliomyelitis vaccine, will be used. Another important direction of research in relation to poliomyelitis is the search for stable strains of *normal* tissue which can be propagated indefinitely in tissue culture and on which poliovirus for use in vaccine manufacture can be grown.

Tissue culture studies have prompted an increasing number of reports of poliomyelitis-like diseases. A diagnosis commonly given is aseptic meningitis, which cannot be distinguished clinically from *nonparalytic* poliomyelitis. Tissue culture studies have associated ECHO and Coxsackie viruses with outbreaks of poliomyelitis-like diseases.

The possibility for fuller rehabilitation of patients stricken with paralytic poliomyelitis has become increasingly evident in recent years. The National Foundation for Infantile Paralysis is supporting and encouraging a number of programs aimed in this direction; for example, 15 respiratory and rehabilitation centers.

The hope of eliminating paralytic poliomyelitis lies in the widest

possible vaccination against it. It is to be anticipated that pediatricians will play a leading role in reaching this goal, just as they have in conquering other diseases through immunization procedures.

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